#### ANTIMICROBIAL COMPOSITIONS

#### REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from provisional application Serial Nos. 60/407,050, filed August 30, 2002; 60/441,384, filed January 21, 2003; 60/441,584, filed January 21, 2003; 60/456,673, filed March 21, 2003; 60/456,732, filed March 21, 2003; and 60/465,549, filed April 25, 2003; which are all hereby incorporated by reference in their entirety.

#### FIELD OF THE INVENTION

5

10

15

20

25

30

The present invention generally relates to the [0002] provision of an antimicrobial composition and of methods of controlling the growth of, preventing the growth of, or killing, microbial organisms. The present invention also generally relates to methods for inhibiting or killing microbes in water and food, including human food, livestock food, pet food, or other animal food; methods for inhibiting or killing mold in water and food; methods for inhibiting or killing bacteria in water and food; methods for inhibiting bacteria in water and food; methods for inhibiting mold in water and food; methods for killing bacteria in water and food; methods for killing mold in water and food; methods for delaying formation of mold in water and food; methods for enhancing digestibility and/or palatability of water and food; and methods of delaying or stopping mold growth in water and food.

#### BACKGROUND OF THE INVENTION

[0003] Control of bacteria in animal feed is an ongoing challenge for the industry, even more so as consumers focus on the health and safety of the meat they feed their families. Control of bacteria is desirable to protect the health of the animal for which the feed is intended. Gut bacteria in the animal compete for nutrients and can be detrimental to the health and performance of the

animal. Second, and of growing importance, the reduction of bacteria in feed is an important part in the reduction and control of food borne diseases.

5

10

15

20

25

30

35

[0004] The use of antibiotics in animal feed has recently come under scrutiny, in part due to concerns that overuse of antibiotics may result in resistance to the antibiotics in the animals treated. Additionally, upon consumption of animals treated with antibiotics, humans may experience problems due to allergies to the antibiotics, or in becoming resistant to the antibiotics as well. Thus, a need exists for alternative means of inhibiting bacteria in animal feed.

[0005] Two main alternatives are currently available for control of bacteria in feed: thermal and chemical methods. Heat treatment of feed is costly, but efficient. However, heat treatment alone does not avoid recontamination of the feed between the time the feed is treated and consumed by the animal.

[0006] Chemical treatments primarily involve the use of formaldehyde or an organic acid to the feed. Formaldehyde has been used extensively in the UK, although its use remains under scrutiny by the EU Commission.

[0007] Organic acids, on the other hand, have many applications in the animal feed industry worldwide. For many years, nutritionists have used organic acids in piglet diets for their positive effect on health and growth. For instance, formic acid is used to decontaminate raw materials, and propionic acid is used to control mold. Other organic acids commonly used include fumaric, citric and lactic acids. Organic acids are also known to modify the gastrointestinal flora.

[0008] Approvals for the use of non-therapeutic antibiotics in animal feed have been withdrawn in several countries and the practice of feeding low-dose growth promoters is fast disappearing worldwide. Already, producers who want to sell in the drug-free market are searching for non-pharmaceutical replacements for feed

antibiotics. Most data suggest that the growth-promoting effect of antibiotics can be entirely ascribed to their antimicrobial activity and the physiological repercussions from that. Thus, the search for replacements has focused on naturally occurring molecules with known anti-microbial activity.

5

10

15

20

25

30

35

The primary effect of antibiotics is antimicrobial; all of the digestibility and performance effects can by explained by their impact on the gastrointestinal microflora and the resulting reduction in immune stimulation. Organic acids have antimicrobial activity; however, there appear to be effects of organic acids beyond those attributed to antimicrobial activity. Reductions in certain species of bacteria are associated with feeding organic acids, which are particularly effective against acid intolerant species like E. coli, Salmonella and Campylobacter. Both antibiotics and organic acids improve protein and energy digestibility by lowering the incidence of background immune stimulation and the resulting synthesis and secretion of immune mediators, by reducing production of ammonia and other growth-depressing microbial metabolites and perhaps by reducing the overall microbial load. Unlike antibiotics, the antimicrobial activity of organic acids is pH dependent. Organic acids have a clear and significant benefit in weanling piglets, and have been observed to benefit poultry performance. Organic acids have several additional effects that go beyond those of antibiotics. These include reduction in digesta pH and increased pancreatic secretion.

[0010] Pigs are susceptible to weaning stresses (separation from sows, environmental changes, and physical effects of solid feed) and a variety of pathogens such as E. coli and rotavirus. These pathogens are reduced in adult animals by the reduction of pH in the stomach, but young pigs have lower hydrochloric acid secretion from the stomach. In addition, the failure to acidify gastric contents coupled with low pancreatic enzyme secretion can

lead to insufficient mutrient nine to enteric diseases lead to insutticient nutrient digestion and also increases.

lead to insutticient nutrient pigs to enteric diseases.

the susceptibility of weaning pigs have domimented the susceptibility of atualies have domimented the susceptibility. NUBCEPTIDILITY OF WEARING PIGS to enceric disease, 100111 A number of studies have documented the (UUIL)

A number of studles have documented swine,

a nave documented swine,

a nave documented swine,

a recent minimation

effects of organic acids on performance in recent minimation

effects of organic meaned minimate a recent minimation. effects of organic acids on performance in young swine, by weaned piglets. A recent publication by effects of organic acids on piglets. A recent modes of modes of piglets. Their efficacy and modes of particularly early acids. Their efficacy and modes of particularly acids. particularly weared piglets. A recent publication of their efficacy and modes of their efficacy and modes of their efficacy and modes of their partanen ("Organic acids: particularly early weared piglets. A recent publication of particularly weared piglets. A recent piglets of particularly weared piglets of particularly weared piglets. A recent piglets of particularly weared piglets of particularly weared piglets. A recent piglets of particularly weared Partanen ("Organic acids: their efficacy and modes of piva, university press, Nottingham, university press, Nottingham university pr action in pigs, he literature in this area and nrowides

A. et al., reviews the literature in this area. A. et al. eds, Nottingham University Press, and Provides

(2001)) reviews mers analyze of evicting data (2001) reviews (2001) reviews the literature in this area and provide the results of a meta-analysis of the results individual acide in the sheep of the results of individual acide in the sheep of the results of individual acide in the sheep of the results of individual acide in the sheep of the results of individual acide in the sheep of the results of individual acide in the sheep of the results of individual acide in the sheep of the results of the studies using individual acids in the absence of In the analysis of rrials are considered. The armificant are considered trials and copper are fartaning night and 23 fartaning night and 23 fartaning night and 23 fartaning night. the results of a meta-analysis of existing data. (

the results of a meta-analysis of the absence of individual acids in the absence of studies using individual acids in the absence of the results of a meta-analysis of existing data. ( antibiotics and copper are considered. In the analysis of trials, significant trials, fumaric.

antibiotics and copper are considered. In the analysis of trials, fumaric.

food-to-main improvements were seen with formic. fumaric. 46 Weaned piglet and 23 fattening pig trials, significant weight formic, weight formate were seen with calcium diformate weight feed-to-gain improvements with calcium diformate and also with calcium diformate weight feed-to-gain acide and also with calcium diformate. feed-to-gain improvements were seen with tormic weight calcium diformate. Weight and citric acids and affects were significant for formic and citric acids and intake effects were significant for formic and citric acids and intake effects were significant for formic and citric acids and intake effects were significant formic. and citric acids and also with calcium diformate. Weight to formic and citric acids and effects were significant that dietary and gain and feed intake the author concludes that dietary acid and diformate 5 gain and feed intake effects were significant for formic that dietary acids

meaned nicial and diformate. The author concludes were significant for formic that dietary acids that dietary acids that dietary acids that dietary acids and feed intake author concludes were significant for formic that dietary acids are a meaned nicial acid and diformate. acid and diformate. The author concludes that dietary aci that is primarily associated with changes in the al., I.

that is primarily associated with changes in the al., I.

gastrointestinal microflora. see Eidelsburger et al., I.

gastrointestinal microflora. sa. a2-92 (1992)

Anim Anim nave a peneticial effect; especially on weaned pictory associated with changes in the that is primarily associated with night of the that is primarily migration. 10 Envelor Without being limited to a particular theory;

[0012] Without one merhaniam for the artion of organian to the particular theory;

[0012] Without one merhaniam for the artion of organian theory; [0012] Without being limited to a particular theory in the action of organic without being limited for the action changes the action of organic when action of organic the action of organic when action of organic the action of organic without being limited to a particular theory in the action of organic when action of organic when action of organic when action of organic theory is action of organic the action of organic theory is action of the action of organic theory is action of the action of organic the action of yabulullar microrrora. See Elaelsourger

Anim. physiol. Without haird limited to a north it is believed that one mechanism for the action of changes the acids changes the wirh it and antimicrohial acids as antimicrobials in accordance with it and antimicrohial acids as antimicrohial in accordance with its antimicrohial acids as antimicrohial in accordance with its antimicrohial acids as antimicrohial acids acid acids as antimicrobials is as tollows. The acid changes the with its antimicrobial in accordance with its rhe change in microbial populations once inside the cell the change in microbial populations once inside the cell the change in microbial populations once inside the cell the change in microbial populations of activity. microbial populations in accordance with its antimicrobial the change in once inside the cell, once inside weak acide where the disconstitution of the property of activity. Once inside weak acide weak acide where weak acide the cell, once inside weak acide where we aci 15 spectrum or activity. Once inside the cell, the change weak acids. The ph causes the dissociation of these weak acids. multiple effects of organic acids are due to this to it.

multiple effects of organic acids are due to response to it.

intracellular dissociation and the cellular result of the deleterious intracellular activity is a result of the deleterious activity in the deleterious activity is a result of the deleterious activity in the deleterious activity is a result of the deleterious activity in the deleterious activity is a result of the deleterious activity in the deleterious activity is a result of the deleterious activity in the deleterious activity is a result of the deleterious activity in the deleterious activity is a result of the deleterious activity in the deleterious activity is a result of the deleterious activity in the deleterious activity is a result of the deleterious activity. ph causes the dissociation of these weak acids. The multiple effects of organic acids are due to this The antimicrobial activity is a result of the free anion on the free proton and the firee proton and the effects of the fundal cell the hacterial or fundal cell 20 effects of the free proton and, perhaps, the avnrheaia of the bacterial or rhowards to reall. the bacterial or rungal cell. In the gut enterocytes, of the bacterial or rungal to result in the synthesis of thought to result in anormal to result in the synthesis of the bacterial or rungal cell. dissociation is thought to result in the synthesis of result in the synthesis of secretion.

dissociation is thought to result in the synthesis secretion.

herefite that a herefite that an herefite that are the herefite that an Thus, organic acids have benefits that go beyond to control the activity nominations antimicrobial activity. While in the mit, the nominates while in the mit, the nominates while in the mit. 25 secretin, a normone that stimulates pancreatic secretin, a normone that benefits that go beyond the order. antimicrobial activity. For feeds, the gut, the populations fungal growth dominates, 30 35

being affected are primarily the bacteria whose growth is much affected by acidic conditions. It should be most affected by acidic conditions. Deing affected by acidic conditions. It should be most affected by acidic the mechanism of acrism and however that the mechanism of acrism affected by acidic the mechanism of acrism of a most affected by acidic conditions. It should be of action of the mechanism of action of that the mechanism of in addition of the mechanism of in addition of the mechanism of action of actio emphasized, however, that the mechanism of action or that the mechanism and in addition to action of that the mechanism of action of action to and in addition to another that of increasing acids is quite such as well acids is acids is acids in acids acids is acids acids is acids acids is acids that of inorganic acids such as HCl. see, e.g., low pH on the importance of low pH on organic acids is quite different from and in addition of inorganic acids such as HCl. see! Eidelsburger et al., supra. The importance of low ph on the more acids can be explained by nH. more acids can be explained more acids acid. At low nH. more antimicrobial activity of organic of the acid. At low nH. more antimicrobial rhe diseaseign of the acid. antimicrobial activity of organic acids can be explained by

antimicrobial activity of organic acids acid. At low pH, more

its effect on the dissociation of the undissociated form

its effect on acid will be in the undissociation of the undissociation the und Its effect on the dissociation of the undissociated form.

Its effect on the dissociated will be in the undissociated and distributed the organic acid will be in the property and the undissociated form. or the organic acids are lipophilic and can diffuse indissociated organic acids are three of harraria and undissociated organic acids are three of harraria and undissociated organic acids are three of harraria and undissociated organic acids are three of harraria and the organic acids are three of harraria and undissociated organic acids are three of harraria and undissociated organic acids are three organic ac undissociated organic acids are lipophilic and can diffu those of bacteria and including those of bacteria and including those of party including molas. See huyghebaert, Report: CLO-DVV (1999) and cell, the bacterial cell, once in the bacterial rhe acid are on a supra. Once in the bacterial rhe acid are on a supra. Causea diagoniation of the acid are on a supra. Eidelaburger et al., ovt.onlasm causea diagoniation of the higher by of its cyt.onlasm across cell memoranes, across see Huyghebaert, Report: CLO-DVV (1999) and molds. See Huyghebaert and across see Huyghebaert across see H Eidelsburger et al., supra. once in the bacterial cell, the acid, once in the bacterial of the acid, will higher ph of its cytoplasm in nh of the cell contents will higher the resulting reduction in and the resulting reduction. nigher ph of its cytoplasm causes dissociation of the acid will night reduction in ph of the resulting reduction of anyumaric reaction and the resulting of disruprice of and the referra of disruprice of have and the resulting reduction in pH of the cell contents and have the effects of disruption can charring of the reactions have the transport avarame can have the transport avarame 5 have the effects of disruption of enzymatic reactions and the process of disruption of enzymatic reactions. nutrient transport systems. See Cherrington et al., Adv. the process [1991]. In addition, a energy 32:87-108 [1991]. In addition, a energy [1991]. In additi Microb. Physio., the free proton out of the energy of transporting and this contributes to reduced energy of transporting and the contributes to reduced energy. requiring and this contributes to reduced energy degree of reduced energy in some degree of resulting in some degree of resulting and this contributes resulting activity is activity and this contributes antimicrohial activity and direct antimicrohial activity availability for main direct antimicrohial activity availability and activity and direct antimicrohial activity availability availability. 10 or cransporting the free proton out of the energy requiring and this contributes to reduced energy avallability for proliferation, resulting in some degree antimicrobial activity is bacteriostasis. pacterlostasis. This direct antimicrobial activity is pacterlostasis. This direct for feed and food sanitation are not responsible to the use of organic activity is antimicrobial activity is pacterlostasis. This direct antimicrobial activity is pacterlostasis. This direct antimicrobial activity is pacterlostasis. believed to be responsible to the use of organic acids as effects that contribute Most food production animals especially pigs 15 100131 Most food production animals, especially pi

Nost food production animals, especially pi

require supplemental methionine
renroduction

renroduction

renroduction

renroduction

poultry and cattle, require arowth and renroduction

their diets for proper arowth and renroduction poultry and cattle, require supplemental methionine in trade their diets for proper acid (HMMPA) sold under the trade their diets for proper acid (HMMPA) their diets for proper growth and reproduction. 2-hydroxy

their diets for proper growth and reproduction the trade

4-(methylthio) butanoic Thramational Thramational

4-(methylthio) hy Norma 4-Imethylthio) butanoic acid (HMBA, sold under the trade of elimiemental methic)

name Alimet® by Novus acuirca of elimiemental methic)

Microurill is a nomilar acuirca of elimiemental methic) name Alimet by Novus International, Inc. (St. Louis, Inc. 20 Missouri)) is a popular source of supplemental methionine warring has become a preferred source of supplemental methionine (nTM) for feed mills warring for animal diets. preservatives. for animal diets. Alimet has become a preterred wanting to (DLM), for feed mills wanting to over powder d.l-methionine associated with increased solve the common problems associated with increased over powder d,1-metnionine associated with increased solve the common problems associated with increased 25 the physical form of liquids. Easier bulk handling with its disposal of packaging with its accurate dosage. production capacity because of the efficiency advant the physical form of liquids. Fasier with its the physical form of liquids. 30 35

and the inventory shrink issues, as well as dust reduction, are popular features. Alimet® contains 88% methionine activity, while liquid DLM contains only 40% methionine activity. This low level of relative activity in DLM means water takes the place of valuable energy and protein components in the feed, reducing nutrient density.

5

10

15

20

25

30

35

[0014] Preventing or delaying the growth of mold in animal feed compositions is beneficial, in that less feed is lost to spoilage, and illnesses associated with the molds or toxins they produce can be avoided.

Mold in feed rations can render the feed unfit [0015] for consumption. Moldy feed may decrease the digestibility and/or palatability of the feed, both of which can adversely affect production and health of the animal. Additionally, many molds produce mycotoxins which affect the nutrient value of feed, or which may be hazardous to the health of animals, including livestock and humans. Aspergillus aflatoxin B, mycotoxin is a potent liver carcinogen; certain Penicillium mycotoxins affect liver or kidney function; and Fusarium mold species are associated with pulmonary edema in swine, liver cancer in rats, and abnormal bone development in chicks and pigs. United States Department of Agriculture, "Grain Fungal Diseases & Mycotoxin Reference, " available at http://www.usda.gov/qipsa/pubs/pubs.htm. Pigs are particularly sensitive to the presence of Fusarium mycotoxins, especially deoxynivalenol (DON), also known as vomitoxin.

[0016] Detoxifying feed which has been contaminated with mycotoxins can be quite difficult, and often is accomplished only by subjecting the feed to extreme processing conditions. For example, corn contaminated with aflatoxin, a mycotoxin produced by the Aspergillus species of mold, can be detoxified by treating the corn under pressure with hot, moist ammonia. Thus, the need exists for a way to prevent formation of mold and the mycotoxins they may produce.

[0017] Mold growth in untreated, stored feeds is wigher Note a growth in untreated, stored reeds is humid conditions. Higher humid conditions around around around in hot, humid for mold around heart in heart the change for mold around heart increase the temperatures increase the chance for mold growth, in the with high moisture levels in araina particularly when feed mold growth rarely ordinated with particularly when feed mold growth rarely ordinated mold growth rarely ordinated to chance for mold growth rarely ordinated in array ordinated feed mold growth rarely ordinated feed mold growth. especially prevalent in not numid conditions. High particularly when coupled with high moisture levels in grains feed. In chicken than 14-15% moisture However. even feed. Than 14-15% moisture. However. even feed. Then the containing less than 14-15% moisture. teed. In chicken than 14-15% moisture. However, with containing less than average moisture level may have nockers with with a low average moisture. containing less than 14-15% moisture. However, even reeds with level may have pockets with a low average moisture level may microclimates ideal for with a low average producing microclimates ideal for with a low average producing microclimates. With a low average moisture level may have pockets with may have pockets ideal for microclimates ideal for microclimates in warmer climates.

high moisture this often hannens in warmer climates.

high moisture this often hannens in warmer climates. nigh molsture levels, producing microclimates, climates, and molsture This often happens in warmer the first time temperature and night time temperature and night time temperature. mold growth. This orten nappens in warmer climates, temperatures cause temperatures cause temperatures cause temperatures. especially where cool night time temperatures cause feed is where grains or feed is bins where grains or mond growth time temperatures cause feed is time temperatures cause feed is the condensation inside silos or bins where grains or mond grand condensation inside relatively amail nookers of mond grand condensation these relatively amail nookers of mond grand gra condensation inside silos or pins where grains or reed is where grains or mold growth pockets of mold growth he nrohiematic to hirds stored. Even these relatively small pockets he nrohiematic to hirds stored. stored. Even these relatively small pockets of mold growth a problematic normanced to birds of mycotoxins can be problematic; mycotoxins for trientherenes of mold growth problematic to birds of the problematic normanced the problematic; mycotoxins for trientherenes of mold growth problematic to birds of the problematic; mycotoxins for trientherenes of mold growth problematic to birds of the can be problematic; mycotoxins can be problematic to birds tricothecenes, produced tricothecenes, produced by the at parts-per-million levels (e.g., and aflatoxins. produced by the fugarium species. at parts-per-million levels (e.g., tricothecenes, produced by the by the fusarium species, and aflatoxins, produced by the by the fusarium anerical glius species). that certain feed additives LUULUI LE LS KNOWN that certain reed additives bind act as antifungals) or bind when act as antifungals in and when are notion when and mold growth (i.e., additives are notion additives are notion when the contract of the prevent mold growth (i.e., act as antifungals) or bind act as antifungals) or where such additives are indicationally existing mycotoxins. existing mycotoxins. Such additives are often used where such additives are often used is logistically feed is logistically in mixed feed is logistically inclination of mixed feed is logistically inclination. 10 moleture control in grain or mixed reed is logistically including difficult. The use of certain organic acids, in the difficult. Aspergillus species). difficult. The use of certain organic acids, including acids, including acids, is known.

difficult. The use of certain organic acids is known.

acid, as mold inhibitors is known.

acids, including acids, include aluminositicates

acid, as mold inhibitors is known. Propionic and acetic acid, as mold inhibitors is known.

Adsorbents to bind mycotoxins include aluminosilicates and bind mycotoxins include armine allow the aluminosilicates and include aluminosilicates aluminosilicates and include aluminosilicates Ausorpence to pina mycotoxins include aluminosilicates the minor allow the whose chemical structure are minor and products; and perhaps other minor and clay loam products; and perhaps other minor and capture of aflatoxin. capture of aflatoxin, have variable efficacy, of a separate mycotoxin have variable efficacy, of a separate where the contract of a separate of aflatoxin have variable efficacy. The of a separate of aflatoxin characteristics. clay loam products, whose chemical structure allow the capture of aflatoxin, have road of a flatoxin, have road on the capture of a flatoxin, have read on the capture 15 Mycotoxin adsorbents have variable ettlcacy; of a separate their variable binding characteristics. Use their increases the coat adsorbent adsorbent increases the coat and their variable binding characteristics. their variable binding characteristics. Use or a separ the costs adsorbent increases the costs and inhibitor or mold adsorbent increases the costs and inhibitor or mold feed 100191 A previous study (Doerr et al., Poultry 20 LOUISI A previous study (Doerr et al., Poultry may suggested that Alimet® may 1995)) suggested that Alimet® may 3 (1995)) suggested that Alimet® may 5 (1995) suggested that Alimet® may 6 (1995) suggested that Alimet® may 7 (1995) suggested that Alimet® may 8 (1995) suggested that Alimet® may 9 (1995) suggested that 1995 suggested that 1995 suggested that 1995 suggested that 1995 suggeste Science the growth of mold in samples of sabouraud's reduce reduce the growth ot mold in samples of sabouraud's (19% or ground corn or mold in samples or ground corn or mold in samples or ground corn or mold in samples of sabouraud's (19% or ground corn or ground corn or mold in samples of sabouraud's (19% or ground corn or ground cor associated with animal feed. dextrose protn, potato dextrose agar, or ground corn (1)

dextrose protn, potato dextrose agar, or ground corn (1)

either Aspergillus parasiticus, and in monitiforms and in moisture)

treated with or rise arium monitiforms

agar, or ground corn (1)

a Aspergillus ochraceus, around corn (17.5% moiatura) with its naturally ochraceus around corn 25 Aspergillus ochraceus, or Fusarium monilitorme, and in occurring or Fusarium monilitorme, occurring with its naturally occurring with its naturally occurring and in or occurring with its naturally occurring ochraceus, or Fusarium monilitorme, and in occurring occurring occurring ochraceus, or Fusarium monilitorme, and its naturally occurring oc 30 fungal flora. 35

[0020] Enhancing the palatability of animal food is an endless endeavor by food manufacturers. Addition of palatants to the food is desirable as a means to increase acceptance by the animals, resulting in improved health of the animal, increased weight gain, etc. Palatants are frequently used in foods for canines, felines, and aquaculture.

### SUMMARY OF THE INVENTION

5

10

15

20

25

30

35

[0021] Accordingly, the present invention provides methods for inhibiting bacteria in animal feed.

[0022] In another aspect, the present invention provides methods for inhibiting mold in food, food ingredients, and animal feed compositions.

[0023] In another aspect, the present invention provides an anti-bacterial composition comprising a compound of Formula I, as defined herein, for use in inhibiting bacteria in animal feed.

[0024] In yet another aspect, the present invention provides an anti-bacterial composition comprising a compound of Formula I and one or more organic acids for use in inhibiting bacteria in animal feed.

[0025] Briefly, therefore, the present invention is directed to an anti-bacterial composition comprising a compound of Formula I, as defined herein.

[0026] The present invention is also directed to an anti-bacterial composition comprising a compound of Formula I and one or more organic acids.

[0027] The present invention is also directed to a method for inhibiting bacteria in animal feed, said method comprising treating said feed with an anti-bacterial composition comprising a compound of Formula I.

[0028] The present invention is also directed to a method for inhibiting bacteria in animal feed, said method comprising treating said feed with an anti-bacterial composition comprising a compound of Formula I and one or more organic acids.

The present invention is also directed to a method for inhibiting bacteria in silage, said method comprising treating said silage with an anti-bacterial composition comprising a compound of Formula I.

The present invention is also directed to a

method for inhibiting bacteria in silage, said method comprising treating said silage with an anti-bacterial composition comprising a compound of Formula I and one or more organic acids.

10

5

The present invention is also directed to a [0031] method for inhibiting bacteria in animal feed comprising computing the concentration in said feed of a compound of Formula I necessary to inhibit bacteria present in said animal feed, and applying said compound of Formula I to said feed in a proportion sufficient to achieve said concentration.

15

[0032] The present invention is also directed to a method for inhibiting bacteria in animal feed comprising heat-treating said animal feed, computing the concentration in said feed of a compound of Formula I necessary to inhibit bacteria present in said animal feed, and applying said compound of Formula I to said feed in a proportion sufficient to achieve said concentration.

25

20

The present invention is also directed to a method for inhibiting bacteria in animal feed comprising computing the concentration in said feed of said compound of Formula I and another organic acid or mixture of organic acids necessary to inhibit bacteria present in said animal feed, and applying said compound of Formula I and said organic acid or mixture of organic acids to said feed in a proportion sufficient to achieve said concentration.

30

35

The present invention is also directed to a method for inhibiting bacteria in animal feed comprising heat-treating said animal feed, computing the concentration in said feed of a compound of Formula I and said organic acid or mixture of organic acids necessary to inhibit bacteria present in said animal feed, and applying said

```
NVI 5252.4
                                                                                         PATENT
compound of Formula I and said organic acid or mixture of an entrient to any feed in a proportion sufficient.
  compound of Formula I and said organic acid or mixture of sufficient to said feed in a proportion sufficient to organic acids to said feed in a proportion sufficient to acids to said representation
                                  Concentration is also directed to a the present invention is also directed to a
```

nethod of method for inhibiting mold in animal feed inhibiting mold in animal feed inhibiting mold in animal feed comprising directly or indirectly making information of a comprising directly or indirectly making in said feed of a recent concentration in said feed of a recent concentration in said feed of a computing the concentration hadrer; a recent concentration hadrer; a recent concentration hadrer; a recent concentration had compared to compared t method of method tor indirectly making information or indirectly making information or indirectly making in early comprising the concentration in early comprising the concentration in early making in early for commuting the concentration in early making in early for commuting the concentration in early making in early for commuting the concentration in early making in early for commuting the concentration in early making in early for commuting the concentration in early making information. available for computing the concentration in said reed or a present to inhibit bacteria and compound of Formula I necessary to hear treated. and compound of Formula which has been hear treated. achieve said concentration. compound of Formula 1 necessary to Innibit pacteria prompound of Formula 1 necessary to heat-treated, and feed which has been heat-treated, and in said animal feed which has been heat-treated, and in said animal feed which has been heat-treated, and in said animal feed which has been heat-treated, and information are also and the said animal feed which has been heat-treated, and information are also and the said animal feed which has been heat-treated, and the said animal feed which has been heat-treated, and the said a in said animal teed which has been neat-treated, and for information available for a said feed in a directly making information as in a directly are information as in a directly are information as information as in a manning of mormula Tro said feed in a directly are information as informat directly or indirectly making information available for a grand of Formula I to said concentration applying said compound of social concentration applying said compound to social concentration available for social concentration availabl

5

10

15

20

25

applying sald compound or Formula 1 to sald reed in a concentration.

The proportion sufficient proportion is a proportion of the proporti retion sufficient to achieve said concentration. to a concentration is also directed to a invention is a concentration.

[10036] nethod of method direction and method of method of method of method of method direction and method of meth comprising directly or indirectly making information in said feed of a computing the concentration in acid or available for computing T and another organic acid or available of Formula T and another organic acid or method of method for commuting the comprising directly or indirectly making information to comprising directly are the comprising the comprision for commuting the comprision of the co

compound of Formula I and another organic acid or bacteria and another organic acids necessary to inhibit indirectly or indirectly or indirectly or indirectly or combination of organic acids and directly or indirectly or combination of animal feed and directly or indirectly or indi available for computing the concentration in said or compound of Formula I and another organic acid or compound of Formula I. combination of organic acids necessary to innibit bacter:

acids necessary to innibit bacter:

to innibit bacter:

acids necessary to innibit bacter:

and directly or indirectly

arid compound

feed, and directly or indirectly

for anniving said compound

present informarion available for anniving said compound

present informarion available for anniving said compound present in said animal teed, and directly or indirectly of of ordanic animal teed, and directly or indirectly or in making information available for applying said compound of available for applying said of organic acid or combination of achieve formula I and said or a proportion sufficient to achieve formula I and feed in a proportion sufficient to achieve Formula I and said organic acid or sufficient to achieve acids to said feed in a proportion sufficient acids to said roncentration.

The present invention is also directed to a [0037]

The present invention is also directed to a method in animal feed, said method in animal feed, with animal feed, with in combination with inhibiting bacteria in combination with method for inhibiting the feed in combination with mean in animal feed in combination with mean in combination with the feed in combination with mean in the feed in combination with mean in the feed in combination with the feed in combination with method in combination with the feed in combina comprising heat-treating the reed in compination with an anti-bacterial common one treating said feed with an anti-bacterial common of Formula T and common of treating said feed with an anti-bacterial common of commo treating said teed with an anti-bacterial composition one or comprising one of comprising a compound of Formula I and comprising a compound of more organic acids said concentration.

organic acids. Invention is directed to a method the feed common it in an animal feed common it in animal feed common it in an animal feed com Lungal The present Invention 18 directed to a mer line present in an animal feed composition, to the of inhibiting mold in an animal feed composition of rorming of rorming of inhibiting mold in an animal feed composition. or inhibiting mold in an animal teed composition, is a said compound of feed commosition commosition method comprising wherein said feed commosition wherein feed commosition. method comprising applying a compound of Formula I to said composition comprises wherein said feed composition feed composition, wherein said feed composition. more organic acids.

The present invention is also directed to a IUU341

The Present Invention is also directed to a feed in an animal feed in an animal feed in an animal of mold in an animal of mold in an animal of method for delaying the common anniving a common in the method commo method for delaying the formation of mold in an animal feec method for the method comprising applying a compound of the method comprising applying a composition. 30 corn and soy.

Formula I to said feed composition, wherein said feed composition comprises corn and soy.

5

10

15

20

25

30

35

[0040] The present invention is also directed to a method of inhibiting the formation of mold in an animal feed composition, the method comprising applying a compound of Formula I to said feed composition, wherein said feed composition has a moisture content of about 17% or less.

[0041] The present invention is also directed to a method for inhibiting mold in silage, said method comprising treating said silage with an anti-fungal composition comprising a compound of Formula I.

[0042] The present invention is also directed to a method for inhibiting mold in silage, said method comprising treating said feed with an anti-fungal composition comprising a compound of Formula I and one or more organic acids.

[0043] The present invention is also directed to a method for inhibiting the growth of mold in an animal feed composition, the method comprising computing the concentration in said feed composition of a compound of Formula I necessary to inhibit the growth of mold in said feed composition; and applying said compound of Formula I to said feed composition in an amount sufficient to achieve said concentration.

[0044] The present invention is also directed to a method for inhibiting the growth of mold in animal feed comprising computing the concentration in said feed of a compound of Formula I necessary to inhibit the growth of mold in said animal feed; and applying said compound of Formula I to said feed in said concentration.

[0045] The present invention is also directed to a method for improving the mold resistance of an animal feed composition, the method comprising discontinuing the use of DL-methionine as a feed supplement; computing the concentration in said feed of a compound of Formula I necessary to inhibit the growth of mold in said animal

feed; and applying said compound of Formula I to said feed in an amount sufficient to achieve said concentration.

[0046] The present invention is also directed to a method for inhibiting mold in animal feed comprising directly or indirectly making information available for computing the concentration in said feed of a compound of Formula I necessary to inhibit mold present in said animal feed; and directly or indirectly making information available for applying said compound of Formula I to said feed in an amount sufficient to achieve said concentration.

5

10

15

20

25

30

35

[0047] The present invention is also directed to method for inhibiting mold in animal feed comprising directly or indirectly making information available for computing the concentration in said feed of a compound of Formula I and another organic acid or mixture of organic acids necessary to inhibit mold present in said animal feed; and directly or indirectly making information available for applying said compound of Formula I and said organic acid or mixture of organic acids to said feed in an amount sufficient to achieve said concentration.

[0048] The present invention is also directed to the use of a compound of Formula I in the manufacture of a nutrient composition for inhibiting mold in animal feed by treating said feed with said nutrient composition.

[0049] The present invention is also directed to the use of a compound of Formula I and one or more organic acids in the manufacture of a nutrient composition for inhibiting mold in animal feed by treating said feed with said nutrient composition.

[0050] The present invention is also directed to a method of inhibiting mold in an animal feed composition, the method comprising monitoring the concentration of methionine supplement in said feed composition, adding additional amounts of said methionine supplement as needed to achieve an anti-mold effective concentration of methionine supplement in said feed composition.

[0051] The present invention is also directed to a method of enhancing the palatability of animal food, particularly dog and cat food, and food for aquaculture.

[0052] Other objects and features will be in part apparent and in part pointed out hereinafter.

# BRIEF DESCRIPTION OF THE DRAWINGS

5

10

15

20

25

30

35

[0053] Figure 1A is a graph illustrating the effect of varying doses (0.108, 0.3, and 0.83 g/L) of formic acid and Alimet® at pH 4.5 and 6.75 on the number of colony forming units of S. enteritidis after 4 hours.

[0054] Figure 1B is a graph illustrating the effect of varying doses (0.108, 0.3, and 0.83 g/L) of formic acid and Alimet® at pH 4.5 and 6.75 on the number of colony forming units of  $E.\ coli$  after 4 hours.

[0055] Figure 1C is a graph illustrating the effect of varying doses (0.108, 0.3, and 0.83 g/L) of formic acid and Alimet® at pH 4.5 and 6.75 on the number of colony forming units of L. plantarum after 6 hours.

[0056] Figure 1D is a graph illustrating the effect of varying doses (0.108, 0.3, and 0.83 g/L) of formic acid and Alimet® at pH 4.5 and 6.75 on the number of colony forming units of *C. jejuni* after 6 hours.

[0057] Figure 2A is a graph illustrating the pH-dependent antibacterial effect of formic acid and Alimet® on the number of colony forming units of S. enteritidis.

[0058] Figure 2B is a graph illustrating the pH-dependent antibacterial effect of formic acid and Alimet® on the number of colony forming units of E. coli.

[0059] Figure 2C is a graph illustrating the pH-dependent antibacterial effect of formic acid and  $Alimet^{@}$  on the number of colony forming units of L. plantarum.

[0060] Figure 2D is a graph illustrating the pH-dependent antibacterial effect of formic acid and Alimet® on the number of colony forming units of *C. jejuni*.

[0061] Figure 3 is a graph illustrating the effect of varying doses (1, 3 and 5 g/L) formic acid and Alimet $^{\otimes}$  on

the number of colony forming units of *S. enteritidis* after 4 hours at pH 4.5 and 6.75.

[0062] Figures 4A and 4B are graphs illustrating the effect of varying doses of a combination of formic acid and Alimet $^{\otimes}$  on the number of colony forming units of S. enteritidis after 4 hours at pH 4.5 and 6.75.

5

10

15

20

25

30

- [0063] Figure 5 is a graph comparing the effects of hydrochloric acid, formic acid, lactic acid, and  $Alimet^{@}$  on the number of colony forming units of  $E.\ coli$  over time, at pH 4 and 7.3.
- [0064] Figure 6 is a graph showing the effect of moisture level on the number of colony forming units of Salmonella in meat meal premix.
- [0065] Figure 7 is a graph showing the percent recovery of Salmonella for different levels of Alimet® in meat meal premix containing 20% moisture.
- [0066] Figure 8 is a graph showing the effect of Alimet® on the number of colony forming units of Salmonella in meat meal premix containing 20% moisture.
- [0067] Figure 9 is a graph illustrating the  $%CO_2$  in the headspace for a starter mash with a moisture level of 16.8% having no DLM or Alimet®; with 0.2% DLM; and with 0.2% Alimet®.
- [0068] Figure 10 is a graph illustrating the  $%CO_2$  in the headspace for a starter mash with a moisture level of 14.8% having no DLM or Alimet®; with 0.2% DLM; and with 0.2% Alimet®.
- [0069] Figure 11 is a graph illustrating the  $%CO_2$  in the headspace for a starter mash with a moisture level of 12.8% having no DLM or Alimet®; with 0.2% DLM; and with 0.2% Alimet®.
- [0070] Figure 12 is a graph illustrating the %CO<sub>2</sub> in the headspace for a starter mash with a moisture level of 14.8% having no DLM or Alimet®; with 0.2% Alimet®; with 2 lb/ton 65% propionic acid plus 0.2% DLM; with 1.5 lb/ton 65% propionic acid plus 0.2% DLM; and with 1.0 lb/ton 65% propionic acid plus 0.2% DLM.

[0071] Figure 13 is a graph illustrating the %CO<sub>2</sub> in the headspace for a starter mash with a moisture level of 14.8% with 2 lb/ton 65% propionic acid; with 1.0 lb/ton 65% propionic acid; and with 1.0 lb/ton 65% propionic acid plus 0.2% Alimet<sup>®</sup>.

[0072] Figure 14 is a graph illustrating the  $%CO_2$  in the headspace for a starter mash with a moisture level of 14.8% with 2 lb/ton 65% propionic acid; with 1.5 lb/ton 65% propionic acid; and with 1.5 lb/ton 65% propionic acid plus 0.2% Alimet<sup>®</sup>.

[0073] Figure 15 is a graph illustrating the  $%CO_2$  in the headspace for a starter mash treated with propionic acid and propionic acid plus 0.2% Alimet<sup>®</sup> with a moisture level of 16.8%, 14.8%, 12.8%.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

5

10

15

20

25

30

[0074] A method has been discovered for inhibiting microbes in animal feed, said method comprising treating said feed with a compound of Formula I.

[0075] Compounds of Formula I have the following structure:

$$R^{1}$$
  $(CH_{2})_{n}$   $CH_{2}$   $CH_{2$ 

[0076] wherein  $R^1$  is an alkyl group having from one to four carbon atoms;

[0077] n is an integer from 0 to 2;

[0078]  $R^2$  is selected from the group consisting of hydroxy, amino,  $-OCOR^3$ , or  $-NHCOR^3$ ;

[0079] and wherein R<sup>3</sup> is an organic acid derivative;

[0080] or a salt thereof.

[0081] The term "organic acid derivative" means a derivative of any suitable organic acid resulting from removal of the carboxyl function from the acid. Preferably, the organic acid has from one to eight carbon atoms. Suitable organic acid derivatives include, but are not

```
limited to, derivatives of formic acid, acetic acid,
         propionic acid, butyric acid, benzoic acid, lactic acid,
         malic acid, tartaric acid, mandelic acid, citric acid,
         fumaric acid, sorbic acid, boric acid, succinic acid,
         adipic acid, glycolic acid, and glutaric acid.
 5
                      Preferably, R1 is methyl, ethyl, propyl
         (including n-propyl and isopropyl), or butyl (including n-
         butyl, sec-butyl, and t-butyl).
                      In a preferred embodiment, the compound of
              [0083]
         Formula I is selected from the following list of compounds:
10
              [0084]
                      1-hydroxy-1-(methylthio)acetic acid;
              [0085]
                      1-hydroxy-1-(ethylthio)acetic acid;
                      1-hydroxy-1-(propylthio) acetic acid;
              [0086]
                      1-hydroxy-1-(butylthio)acetic acid;
              [0087]
                      1-amino-1-(methylthio)acetic acid;
15
              [8800]
              [0089]
                      1-amino-1-(ethylthio)acetic acid;
                      1-amino-1-(propylthio) acetic acid;
              [0090]
                      1-amino-1-(butylthio)acetic acid;
              [0091]
                      1-carboxy-1-(methylthio)acetic acid;
              [0092]
                      1-acetyloxy-1-(methylthio)acetic acid;
20
              [0093]
              [0094]
                      1-propionyloxy-1-(methylthio)acetic acid;
                      1-butyryloxy-1-(methylthio)acetic acid;
              [0095]
                      1-benzoyloxy-1-(methylthio)acetic acid;
              [0096]
                      1-lactoyloxy-1-(methylthio)acetic acid;
              [0097]
                      1-[2-carboxy-2-(hydroxy)propionyloxy]-1-
25
              [0098]
         (methylthio) acetic acid;
                      1-[2-carboxy-1-(hydroxy)propionyloxy]-1-
               [0099]
         (methylthio) acetic acid;
                      1-[2-carboxy-1,2-(dihydroxy)propionyloxy]-1-
               [0100]
         (methylthio) acetic acid;
30
                      1-[hydroxy(phenyl)acetyl]oxy-1-
               [0101]
         (methylthio) acetic acid;
                      1-[2,3-dicarboxy-2-(hydroxy)butyryloxy]-1-
               [0102]
         (methylthio) acetic acid;
```

1-[2-carboxy-1-carboxymethyl-1-

(hydroxy)propionyloxy]-1-(methylthio)acetic acid;

[0103]

```
[0104]
                      1-(3-carboxyacryloyl)oxy-1-(methylthio)acetic
         acid;
                      1-(2,4-pentadienoyloxy)-1-(methylthio)acetic
              [0105]
         acid;
                      1-(2-carboxypropionyloxy)-1-(methylthio)acetic
5
              [0106]
         acid;
              [0107]
                      1-[(4-carboxy)amyloxy]-1-(methylthio)acetic
         acid;
                      1-glycoloyloxy-1-(methylthio)acetic acid;
              [0108]
                      1-glutaroyloxy-1-(methylthio)acetic acid;
              [0109]
10
                      1-formylamino-1-(methylthio)acetic acid;
              [0110]
                      1-acetylamino-1-(methylthio)acetic acid;
              [0111]
                      1-propionylamino-1-(methylthio)acetic acid;
              [0112]
                      1-butyrylamino-1-(methylthio)acetic acid;
              [0113]
                     1-benzoylamino-1-(methylthio)acetic acid;
15
              [0114]
              [0115] 1-lactoylamino-1-(methylthio)acetic acid;
                      1-[2-carboxy-2-(hydroxy)propionylamino]-1-
              [0116]
         (methylthio) acetic acid;
               [0117]
                      1-[2-carboxy-1-(hydroxy)propionylamino]-1-
         (methylthio) acetic acid;
20
                      1-[2-carboxy-1,2-(dihydroxy) propionylamino]-1-
               [0118]
         (methylthio) acetic acid;
                      1-[hydroxy(phenyl)acetyl]amino-1-
               [0119]
         (methylthio) acetic acid;
                      1-[2,3-dicarboxy-2-(hydroxy)butyrylamino]-1-
               [0120]
25
         (methylthio) acetic acid;
                       1-[2-carboxy-1-carboxymethyl-1-
               [0121]
         (hydroxy) propionylamino] -1-(methylthio) acetic acid;
                       1-(3-carboxyacryloyl)amino-1-
               [0122]
          (methylthio) acetic acid;
30
               [0123] 1-(2,4-pentadienoylamino)-1-(methylthio)acetic
         acid;
               [0124] 1-(2-carboxypropionylamino)-1-
          (methylthio) acetic acid;
                       1-[(4-carboxy)amylamino]-1-(methylthio)acetic
35
               [0125]
         acid:
                       1-glycoloylamino-1-(methylthio)acetic acid;
               [0126]
```

```
1-qlutaroylamino-1-(methylthio)acetic acid;
               [0127]
               [0128]
                       1-carboxy-(ethylthio)acetic acid;
               [0129]
                       1-acetyloxy-(ethylthio)acetic acid;
               [0130]
                       1-propionyloxy-(ethylthio)acetic acid;
 5
               [0131]
                       1-butyryloxy-(ethylthio)acetic acid;
               [0132]
                       1-benzoyloxy-(ethylthio)acetic acid;
               [0133]
                       1-lactoyloxy-(ethylthio)acetic acid;
               [0134]
                       1-[2-carboxy-2-(hydroxy)propionyloxy]-
         (ethylthio) acetic acid;
10
                       1-[2-carboxy-1-(hydroxy)propionyloxy]-
               [0135]
         (ethylthio) acetic acid;
               [0136]
                       1-[2-carboxy-1,2-(dihydroxy)propionyloxy]-
         (ethylthio) acetic acid;
               [0137]
                       1-[hydroxy(phenyl)acetyl]oxy-(ethylthio)acetic
15
         acid;
               [0138]
                      1-[2,3-dicarboxy-2-(hydroxy)butyryloxy]-
         (ethylthio) acetic acid;
               [0139]
                       1-[2-carboxy-1-carboxymethyl-1-
         (hydroxy)propionyloxy] - (ethylthio)acetic acid;
20
                      1-(3-carboxyacryloyl)oxy-(ethylthio)acetic
              [0140]
         acid;
              [0141]
                      1-(2,4-pentadienoyloxy)-(ethylthio)acetic
         acid;
                      1-(2-carboxypropionyloxy)-(ethylthio)acetic
              [0142]
25
         acid;
              [0143]
                      1-[(4-carboxy)amyloxy]-(ethylthio)acetic acid;
                      1-glycoloyloxy-(ethylthio)acetic acid;
              [0144]
              [0145]
                      1-glutaroyloxy-(ethylthio)acetic acid;
                      1-formylamino-(ethylthio)acetic acid;
              [0146]
30
              [0147]
                      1-acetylamino-(ethylthio)acetic acid;
                      1-propionylamino-(ethylthio)acetic acid;
              [0148]
              [0149]
                      1-butyrylamino-(ethylthio)acetic acid;
                      1-benzoylamino-(ethylthio)acetic acid;
              [0150]
              [0151]
                      1-lactoylamino-(ethylthio)acetic acid;
35
              [0152]
                      1-[2-carboxy-2-(hydroxy)propionylamino]-
         (ethylthio) acetic acid;
```

```
1-[2-carboxy-1-(hydroxy)propionylamino]-
              [0153]
         (ethylthio) acetic acid;
                      1-[2-carboxy-1,2-(dihydroxy)propionylamino]-
              [0154]
         (ethylthio)acetic acid;
                     1-[hydroxy(phenyl)acetyl]amino-
5
              [0155]
         (ethylthio)acetic acid;
                      1-[2,3-dicarboxy-2-(hydroxy)butyrylamino]-
              [0156]
         (ethylthio)acetic acid;
                      1-[2-carboxy-1-carboxymethyl-1-
              [0157]
         (hydroxy) propionylamino] - (ethylthio) acetic acid;
10
                     1-(3-carboxyacryloyl)amino-(ethylthio)acetic
              [0158]
         acid;
                      1-(2,4-pentadienoylamino)-(ethylthio)acetic
              [0159]
         acid;
                      1-(2-carboxypropionylamino)-(ethylthio)acetic
15
              [0160]
         acid;
              [0161]
                      1-[(4-carboxy)amylamino]-(ethylthio)acetic
         acid;
                      1-qlycoloylamino-(ethylthio)acetic acid;
              [0162]
                      1-glutaroylamino-(ethylthio)acetic acid;
              [0163]
20
                      1-carboxy-(propylthio)acetic acid;
              [0164]
                      1-acetyloxy-(propylthio)acetic acid;
              [0165]
                      1-propionyloxy-(propylthio)acetic acid;
              [0166]
                      1-butyryloxy-(propylthio)acetic acid;
              [0167]
              [0168] 1-benzoyloxy-(propylthio)acetic acid;
25
               [0169] 1-lactoyloxy-(propylthio)acetic acid;
               [0170]
                      1-[2-carboxy-2-(hydroxy)propionyloxy]-
         (propylthio) acetic acid;
                      1-[2-carboxy-1-(hydroxy)propionyloxy]-
               [0171]
          (propylthio) acetic acid;
30
                      1-[2-carboxy-1,2-(dihydroxy)propionyloxy]-
               [0172]
          (propylthio)acetic acid;
                      1-[hydroxy(phenyl)acetyl]oxy-
               [0173]
          (propylthio)acetic acid;
                       1-[2,3-dicarboxy-2-(hydroxy)butyryloxy]-
35
               [0174]
          (propylthio) acetic acid;
```

```
[0175]
                      1-[2-carboxy-1-carboxymethyl-1-
         (hydroxy)propionyloxy] - (propylthio)acetic acid;
                      1-(3-carboxyacryloyl)oxy-(propylthio)acetic
              [0176]
         acid;
                      1-(2,4-pentadienoyloxy)-(propylthio)acetic
5
              [0177]
         acid;
                      1-(2-carboxypropionyloxy)-(propylthio)acetic
              [0178]
         acid;
              [0179]
                      1-[(4-carboxy)amyloxy]-(propylthio)acetic
         acid;
10
                      1-glycoloyloxy-(propylthio)acetic acid;
              [0180]
                      1-glutaroyloxy-(propylthio)acetic acid;
              [0181]
                      1-formylamino-(propylthio)acetic acid;
              [0182]
                      1-acetylamino-(propylthio)acetic acid;
              [0183]
15
              [0184]
                      1-propionylamino-(propylthio)acetic acid;
                      1-butyrylamino-(propylthio)acetic acid;
              [0185]
              [0186]
                      1-benzoylamino-(propylthio)acetic acid;
                      1-lactoylamino-(propylthio)acetic acid;
              [0187]
              [0188]
                      1-[2-carboxy-2-(hydroxy)propionylamino]-
         (propylthio) acetic acid;
20
                      1-[2-carboxy-1-(hydroxy)propionylamino]-
              [0189]
         (propylthio) acetic acid;
                      1-[2-carboxy-1,2-(dihydroxy)propionylamino]-
              [0190]
         (propylthio) acetic acid;
                      1-[hydroxy(phenyl)acetyl]amino-
              [0191]
25
         (propylthio) acetic acid;
                      1-[2,3-dicarboxy-2-(hydroxy)butyrylamino]-
         (propylthio) acetic acid;
                      1-[2-carboxy-1-carboxymethyl-1-
              [0193]
         (hydroxy) propionylamino] - (propylthio) acetic acid;
30
              [0194] 1-(3-carboxyacryloyl)amino-(propylthio)acetic
         acid:
                     1-(2,4-pentadienoylamino)-(propylthio)acetic
              [0195]
         acid;
                       1-(2-carboxypropionylamino)-(propylthio)acetic
35
              [0196]
         acid;
```

```
[0197]
                      1-[(4-carboxy)amylamino]-(propylthio)acetic
         acid:
              [0198]
                      1-glycoloylamino-(propylthio)acetic acid;
                      1-glutaroylamino-(propylthio)acetic acid;
              [0199]
                      1-carboxy-(butylthio)acetic acid;
5
              [0200]
                      1-acetyloxy-(butylthio)acetic acid;
              [0201]
              [0202]
                      1-propionyloxy-(butylthio)acetic acid;
                      1-butyryloxy-(butylthio)acetic acid;
              [0203]
                      1-benzoyloxy-(butylthio)acetic acid;
              [0204]
              [0205]
                      1-lactoyloxy-(butylthio)acetic acid;
10
                      1-[2-carboxy-2-(hydroxy)propionyloxy]-
              [0206]
         (butylthio) acetic acid;
              [0207]
                      1-[2-carboxy-1-(hydroxy)propionyloxy]-
         (butylthio) acetic acid;
                      1-[2-carboxy-1,2-(dihydroxy)propionyloxy]-
15
              [0208]
         (butylthio) acetic acid;
                      1-[hydroxy(phenyl)acetyl]oxy-(butylthio)acetic
              [0209]
         acid;
              [0210]
                      1-[2,3-dicarboxy-2-(hydroxy)butyryloxy]-
         (butylthio) acetic acid;
20
              [0211]
                      1-[2-carboxy-1-carboxymethyl-1-
         (hydroxy) propionyloxy] - (butylthio) acetic acid;
                      1-(3-carboxyacryloyl)oxy-(butylthio)acetic
              [0212]
         acid;
25
              [0213]
                      1-(2,4-pentadienoyloxy)-(butylthio)acetic
         acid;
              [0214]
                      1-(2-carboxypropionyloxy)-(butylthio)acetic
         acid;
                       1-[(4-carboxy)amyloxy]-(butylthio)acetic acid;
              [0215]
                      1-glycoloyloxy-(butylthio)acetic acid;
30
              [0216]
                       1-qlutaroyloxy-(butylthio)acetic acid;
              [0217]
                       1-formylamino-(butylthio)acetic acid;
              [0218]
                       1-acetylamino-(butylthio)acetic acid;
              [0219]
                       1-propionylamino-(butylthio)acetic acid;
              [0220]
                      1-butyrylamino-(butylthio)acetic acid;
              [0221]
35
              [0222]
                       1-benzoylamino-(butylthio)acetic acid;
                      1-lactoylamino-(butylthio)acetic acid;
              [0223]
```

```
1-[2-carboxy-2-(hydroxy)propionylamino]-
              [0224]
         (butylthio) acetic acid;
              [0225]
                      1-[2-carboxy-1-(hydroxy)propionylamino]-
         (butylthio) acetic acid;
                     1-[2-carboxy-1,2-(dihydroxy)propionylamino]-
5
              [0226]
         (butylthio) acetic acid;
              [0227]
                      1-[hydroxy(phenyl)acetyl]amino-
         (butylthio) acetic acid;
              [0228]
                      1-[2,3-dicarboxy-2-(hydroxy)butyrylamino]-
         (butylthio) acetic acid;
10
                      1-[2-carboxy-1-carboxymethyl-1-
              [0229]
         (hydroxy) propionylamino] - (butylthio) acetic acid;
                      1-(3-carboxyacryloyl)amino-(butylthio)acetic
              [0230]
         acid;
                      1-(2,4-pentadienoylamino)-(butylthio)acetic
15
              [0231]
         acid;
                      1-(2-carboxypropionylamino)-(butylthio)acetic
              [0232]
         acid;
                      1-[(4-carboxy)amylamino]-(butylthio)acetic
              [0233]
         acid;
20
              [0234]
                      1-qlycoloylamino-(butylthio)acetic acid;
                      1-qlutaroylamino-(butylthio)acetic acid;
              [0235]
                      2-hydroxy-3-(methylthio)propanoic acid;
              [0236]
                      2-hydroxy-3-(ethylthio)propanoic acid;
              [0237]
                      2-hydroxy-3-(propylthio)propanoic acid;
25
              [0238]
              [0239]
                      2-hydroxy-3-(butylthio)propanoic acid;
                      2-amino-3-(methylthio)propanoic acid;
              [0240]
                      2-amino-3-(ethylthio)propanoic acid;
               [0241]
                      2-amino-3-(propylthio)propanoic acid;
               [0242]
                      2-amino-3-(butylthio)propanoic acid;
30
               [0243]
                      2-carboxy-3-(methylthio)propanoic acid;
               [0244]
                       2-acetyloxy-3-(methylthio)propanoic acid;
               [0245]
                       2-propionyloxy-3-(methylthio)propanoic acid;
              [0246]
                       2-butyryloxy-3-(methylthio)propanoic acid;
               [0247]
                       2-benzoyloxy-3-(methylthio)propanoic acid;
35
               [0248]
                       2-lactoyloxy-3-(methylthio)propanoic acid;
               [0249]
```

```
[0250]
                      2-[2-carboxy-2-(hydroxy)propionyloxy]-3-
         (methylthio) propanoic acid;
              [0251]
                      2-[2-carboxy-1-(hydroxy)propionyloxy]-3-
         (methylthio) propanoic acid;
5
              [0252]
                      2-[2-carboxy-1,2-(dihydroxy)propionyloxy]-3-
         (methylthio) propanoic acid;
                      2-[hydroxy(phenyl)acetyl]oxy-3-
              [0253]
         (methylthio) propanoic acid;
              [0254]
                      2-[2,3-dicarboxy-2-(hydroxy)butyryloxy]-3-
         (methylthio) propanoic acid;
10
              [0255] 2-[2-carboxy-1-carboxymethyl-1-
         (hydroxy) propionyloxy] - 3 - (methylthio) propanoic acid;
                      2-(3-carboxyacryloyl)oxy-3-
              [0256]
         (methylthio) propanoic acid;
                      2-(2,4-pentadienoyloxy)-3-
15
              [0257]
         (methylthio) propanoic acid;
              [0258]
                      2-(2-carboxypropionyloxy)-3-
         (methylthio) propanoic acid;
              [0259]
                      2-[(4-carboxy)amyloxy]-3-(methylthio)propanoic
20
         acid;
                      2-glycoloyloxy-3-(methylthio)propanoic acid;
              [0260]
                      2-glutaroyloxy-3-(methylthio)propanoic acid;
              [0261]
              [0262]
                      2-formylamino-3-(methylthio)propanoic acid;
                      2-acetylamino-3-(methylthio)propanoic acid;
              [0263]
25
              [0264]
                      2-propionylamino-3-(methylthio)propanoic acid;
                      2-butyrylamino-3-(methylthio)propanoic acid;
              [0265]
              [0266]
                      2-benzoylamino-3-(methylthio)propanoic acid;
                      2-lactoylamino-3-(methylthio)propanoic acid;
              [0267]
              [0268]
                      2-[2-carboxy-2-(hydroxy)propionylamino]-3-
         (methylthio) propanoic acid;
30
              [0269]
                      2-[2-carboxy-1-(hydroxy)propionylamino]-3-
         (methylthio) propanoic acid;
                      2-[2-carboxy-1,2-(dihydroxy) propionylamino]-3-
              [0270]
         (methylthio) propanoic acid;
                      2-[hydroxy(phenyl)acetyl]amino-3-
35
               [0271]
         (methylthio) propanoic acid;
```

```
2-[2,3-dicarboxy-2-(hydroxy)butyrylamino]-3-
         (methylthio) propanoic acid;
                      2-[2-carboxy-1-carboxymethyl-1-
              [0273]
         (hydroxy) propionylamino] -3- (methylthio) propanoic acid;
5
                      2-(3-carboxyacryloyl)amino-3-
              [0274]
         (methylthio) propanoic acid;
                      2-(2,4-pentadienoylamino)-3-
              [0275]
         (methylthio) propanoic acid;
              [0276] 2-(2-carboxypropionylamino)-3-
10
         (methylthio) propanoic acid;
                      2-[(4-carboxy)amylamino]-3-
              [0277]
         (methylthio) propanoic acid;
                      2-qlycoloylamino-3-(methylthio)propanoic acid;
              [0278]
                      2-glutaroylamino-3-(methylthio)propanoic acid;
              [0279]
                      2-carboxy-3-(ethylthio)propanoic acid;
15
              [0280]
                      2-acetyloxy-3-(ethylthio)propanoic acid;
              [0281]
                      2-propionyloxy-3-(ethylthio)propanoic acid;
              [0282]
                      2-butyryloxy-3-(ethylthio)propanoic acid;
              [0283]
                      2-benzoyloxy-3-(ethylthio)propanoic acid;
              [0284]
                      2-lactoyloxy-3-(ethylthio)propanoic acid;
20
              [0285]
              [0286]
                      2-[2-carboxy-2-(hydroxy)propionyloxy]-3-
         (ethylthio) propanoic acid;
              [0287]
                      2-[2-carboxy-1-(hydroxy)propionyloxy]-3-
         (ethylthio) propanoic acid;
25
              [0288]
                      2-[2-carboxy-1,2-(dihydroxy)propionyloxy]-3-
         (ethylthio)propanoic acid;
              [0289]
                      2-[hydroxy(phenyl)acetyl]oxy-3-
         (ethylthio)propanoic acid;
              [0290]
                      2-[2,3-dicarboxy-2-(hydroxy)butyryloxy]-3-
         (ethylthio)propanoic acid;
30
              [0291]
                      2-[2-carboxy-1-carboxymethyl-1-
         (hydroxy) propionyloxy] -3-(ethylthio) propanoic acid;
              [0292]
                      2-(3-carboxyacryloyl)oxy-3-
         (ethylthio) propanoic acid;
                      2-(2,4-pentadienoyloxy)-3-(ethylthio)propanoic
35
         acid;
```

```
2-(2-carboxypropionyloxy)-3-
              [0294]
         (ethylthio) propanoic acid;
                      2-[(4-carboxy)amyloxy]-3-(ethylthio)propanoic
              [0295]
         acid:
                      2-glycoloyloxy-3-(ethylthio)propanoic acid;
5
              [0296]
                      2-glutaroyloxy-3-(ethylthio)propanoic acid;
              [0297]
                      2-formylamino-3-(ethylthio)propanoic acid;
              [0298]
                      2-acetylamino-3-(ethylthio)propanoic acid;
              [0299]
                      2-propionylamino-3-(ethylthio)propanoic acid;
              [0300]
                      2-butyrylamino-3-(ethylthio)propanoic acid;
              [0301]
10
                      2-benzoylamino-3-(ethylthio)propanoic acid;
              [0302]
                      2-lactoylamino-3-(ethylthio)propanoic acid;
              [0303]
                      2-[2-carboxy-2-(hydroxy)propionylamino]-3-
              [0304]
         (ethylthio)propanoic acid;
                      2-[2-carboxy-1-(hydroxy)propionylamino]-3-
15
              [0305]
         (ethylthio) propanoic acid;
              [0306]
                      2-[2-carboxy-1,2-(dihydroxy)propionylamino]-3-
         (ethylthio) propanoic acid;
                      2-[hydroxy(phenyl)acetyl]amino-3-
              [0307]
         (ethylthio)propanoic acid;
20
                      2-[2,3-dicarboxy-2-(hydroxy)butyrylamino]-3-
              [8080]
         (ethylthio)propanoic acid;
                      2-[2-carboxy-1-carboxymethyl-1-
              [0309]
         (hydroxy)propionylamino]-3-(ethylthio)propanoic acid;
              [0310] 2-(3-carboxyacryloyl)amino-3-
25
         (ethylthio)propanoic acid;
                      2-(2,4-pentadienoylamino)-3-
              [0311]
         (ethylthio)propanoic acid;
                      2-(2-carboxypropionylamino)-3-
              [0312]
         (ethylthio)propanoic acid;
30
                      2-[(4-carboxy)amylamino]-3-
              [0313]
         (ethylthio) propanoic acid;
                      2-qlycoloylamino-3-(ethylthio)propanoic acid;
              [0314]
                      2-qlutaroylamino-3-(ethylthio)propanoic acid;
              [0315]
              [0316] 2-carboxy-3-(propylthio)propanoic acid;
35
              [0317]
                      2-acetyloxy-3-(propylthio)propanoic acid;
              [0318]
                      2-propionyloxy-3-(propylthio)propanoic acid;
```

```
2-butyryloxy-3-(propylthio)propanoic acid;
              [0319]
                      2-benzoyloxy-3-(propylthio)propanoic acid;
              [0320]
                      2-lactoyloxy-3-(propylthio)propanoic acid;
              [0321]
                      2-[2-carboxy-2-(hydroxy)propionyloxy]-3-
              [0322]
         (propylthio) propanoic acid;
5
                      2-[2-carboxy-1-(hydroxy)propionyloxy]-3-
              [0323]
         (propylthio) propanoic acid;
              [0324]
                      2-[2-carboxy-1,2-(dihydroxy)propionyloxy]-3-
         (propylthio) propanoic acid;
                      2-[hydroxy(phenyl)acetyl]oxy-3-
10
              [0325]
         (propylthio) propanoic acid;
                      2-[2,3-dicarboxy-2-(hydroxy)butyryloxy]-3-
              [0326]
         (propylthio) propanoic acid;
              [0327]
                      2-[2-carboxy-1-carboxymethyl-1-
         (hydroxy) propionyloxy] - 3 - (propylthio) propanoic acid;
15
                      2-(3-carboxyacryloyl)oxy-3-
              [0328]
         (propylthio) propanoic acid;
                      2-(2,4-pentadienoyloxy)-3-
              [0329]
         (propylthio) propanoic acid;
                     2-(2-carboxypropionyloxy)-3-
20
              [0330]
         (propylthio) propanoic acid;
                      2-[(4-carboxy)amyloxy]-3-(propylthio)propanoic
              [0331]
         acid;
              [0332]
                      2-glycoloyloxy-3-(propylthio)propanoic acid;
25
              [0333]
                      2-glutaroyloxy-3-(propylthio)propanoic acid;
                      2-formylamino-3-(propylthio)propanoic acid;
              [0334]
              [0335]
                      2-acetylamino-3-(propylthio)propanoic acid;
                      2-propionylamino-3-(propylthio)propanoic acid;
               [0336]
              [0337]
                      2-butyrylamino-3-(propylthio)propanoic acid;
                      2-benzoylamino-3-(propylthio)propanoic acid;
30
              [0338]
                      2-lactoylamino-3-(propylthio)propanoic acid;
               [0339]
                      2-[2-carboxy-2-(hydroxy)propionylamino]-3-
               [0340]
         (propylthio) propanoic acid;
                       2-[2-carboxy-1-(hydroxy)propionylamino]-3-
               [0341]
         (propylthio) propanoic acid;
35
               [0342]
                       2-[2-carboxy-1,2-(dihydroxy)propionylamino]-3-
         (propylthio) propanoic acid;
```

```
[0343]
                      2-[hydroxy(phenyl)acetyl]amino-3-
         (propylthio) propanoic acid;
                      2-[2,3-dicarboxy-2-(hydroxy)butyrylamino]-3-
              [0344]
         (propylthio) propanoic acid;
                      2-[2-carboxy-1-carboxymethyl-1-
 5
              [0345]
         (hydroxy)propionylamino]-3-(propylthio)propanoic acid;
                      2-(3-carboxyacryloyl)amino-3-
              [0346]
         (propylthio) propanoic acid;
              [0347]
                     2-(2,4-pentadienoylamino)-3-
         (propylthio) propanoic acid;
10
              [0348]
                      2-(2-carboxypropionylamino)-3-
         (propylthio) propanoic acid;
              [0349]
                      2-[(4-carboxy)amylamino]-3-
         (propylthio) propanoic acid;
              [0350] 2-glycoloylamino-3-(propylthio)propanoic acid;
15
                      2-glutaroylamino-3-(propylthio)propanoic acid;
              [0351]
              [0352] 2-carboxy-3-(butylthio)propanoic acid;
              [0353]
                      2-acetyloxy-3-(butylthio)propanoic acid;
                      2-propionyloxy-3-(butylthio)propanoic acid;
              [0354]
20
              [0355]
                      2-butyryloxy-3-(butylthio)propanoic acid;
                      2-benzoyloxy-3-(butylthio)propanoic acid;
              [0356]
              [0357]
                      2-lactoyloxy-3-(butylthio)propanoic acid;
                      2-[2-carboxy-2-(hydroxy)propionyloxy]-3-
              [0358]
         (butylthio) propanoic acid;
25
              [0359]
                      2-[2-carboxy-1-(hydroxy)propionyloxy]-3-
         (butylthio) propanoic acid;
                      2-[2-carboxy-1,2-(dihydroxy)propionyloxy]-3-
              [0360]
         (butylthio) propanoic acid;
                      2-[hydroxy(phenyl)acetyl]oxy-3-
              [0361]
30
         (butylthio) propanoic acid;
                      2-[2,3-dicarboxy-2-(hydroxy)butyryloxy]-3-
              [0362]
         (butylthio) propanoic acid;
              [0363]
                      2-[2-carboxy-1-carboxymethyl-1-
         (hydroxy) propionyloxy] -3- (butylthio) propanoic acid;
35
              [0364]
                     2-(3-carboxyacryloyl)oxy-3-
         (butylthio) propanoic acid;
```

```
[0365]
                      2-(2,4-pentadienoyloxy)-3-(butylthio)propanoic
         acid;
               [0366]
                       2-(2-carboxypropionyloxy)-3-
          (butylthio) propanoic acid;
 5
               [0367]
                       2-[(4-carboxy)amyloxy]-3-(butylthio)propanoic
         acid;
               [0368]
                       2-glycoloyloxy-3-(butylthio)propanoic acid;
               [0369]
                       2-glutaroyloxy-3-(butylthio)propanoic acid;
               [0370]
                       2-formylamino-3-(butylthio)propanoic acid;
10
               [0371]
                       2-acetylamino-3-(butylthio)propanoic acid;
               [0372]
                       2-propionylamino-3-(butylthio)propanoic acid;
                       2-butyrylamino-3-(butylthio)propanoic acid;
               [0373]
               [0374]
                       2-benzoylamino-3-(butylthio)propanoic acid;
                       2-lactoylamino-3-(butylthio)propanoic acid;
               [0375]
15
               [0376]
                       2-[2-carboxy-2-(hydroxy)propionylamino]-3-
         (butylthio) propanoic acid;
               [0377]
                       2-[2-carboxy-1-(hydroxy)propionylamino]-3-
         (butylthio) propanoic acid;
               [0378]
                      2-[2-carboxy-1,2-(dihydroxy)propionylamino]-3-
20
         (butylthio) propanoic acid;
                       2-[hydroxy(phenyl)acetyl]amino-3-
               [0379]
         (butylthio) propanoic acid;
               [0380]
                      2-[2,3-dicarboxy-2-(hydroxy)butyrylamino]-3-
         (butylthio) propanoic acid;
25
               [0381]
                      2-[2-carboxy-1-carboxymethyl-1-
         (hydroxy) propionylamino] -3 - (butylthio) propanoic acid;
              [0382]
                      2-(3-carboxyacryloyl)amino-3-
         (butylthio) propanoic acid;
                      2-(2,4-pentadienoylamino)-3-
              [0383]
30
         (butylthio) propanoic acid;
              [0384]
                      2-(2-carboxypropionylamino)-3-
         (butylthio) propanoic acid;
               [0385]
                      2-[(4-carboxy)amylamino]-3-
         (butylthio) propanoic acid;
35
              [0386]
                      2-glycoloylamino-3-(butylthio)propanoic acid;
              [0387]
                      2-glutaroylamino-3-(butylthio)propanoic acid;
              [0388]
                      2-hydroxy-4-(methylthio)butanoic acid;
```

```
2-hydroxy-4-(ethylthio)butanoic acid;
              [0389]
              [0390]
                      2-hydroxy-4-(propylthio)butanoic acid;
                      2-hydroxy-4-(butylthio)butanoic acid;
              [0391]
              [0392]
                      2-amino-4-(methylthio)butanoic acid;
                      2-amino-4-(ethylthio)butanoic acid;
5
              [0393]
                      2-amino-4-(propylthio)butanoic acid;
              [0394]
                      2-amino-4-(butylthio)butanoic acid;
              [0395]
                      2-carboxy-4-(methylthio)butanoic acid;
              [0396]
              [0397]
                      2-acetyloxy-4-(methylthio)butanoic acid;
10
              [0398]
                      2-propionyloxy-4-(methylthio)butanoic acid;
              [0399]
                      2-butyryloxy-4-(methylthio)butanoic acid;
                      2-benzoyloxy-4-(methylthio)butanoic acid;
              [0400]
                      2-lactoyloxy-4-(methylthio)butanoic acid;
              [0401]
                      2-[2-carboxy-2-(hydroxy)propionyloxy]-4-
              [0402]
         (methylthio) butanoic acid;
15
              [0403]
                      2-[2-carboxy-1-(hydroxy)propionyloxy]-4-
         (methylthio) butanoic acid;
               [0404]
                      2-[2-carboxy-1,2-(dihydroxy)propionyloxy]-4-
         (methylthio) butanoic acid;
20
               [0405]
                      2-[hydroxy(phenyl)acetyl]oxy-4-
         (methylthio) butanoic acid;
                      2-[2,3-dicarboxy-2-(hydroxy)butyryloxy]-4-
         (methylthio) butanoic acid;
                      2-[2-carboxy-1-carboxymethyl-1-
               [0407]
         (hydroxy) propionyloxy] -4 - (methylthio) butanoic acid;
25
               [0408]
                      2-(3-carboxyacryloyl)oxy-4-
         (methylthio) butanoic acid;
                      2-(2,4-pentadienoyloxy)-4-(methylthio)butanoic
              [0409]
         acid;
30
              [0410]
                      2-(2-carboxypropionyloxy)-4-
         (methylthio) butanoic acid;
              [0411]
                      2-[(4-carboxy)amyloxy]-4-(methylthio)butanoic
         acid;
                      2-glycoloyloxy-4-(methylthio)butanoic acid;
               [0412]
                      2-glutaroyloxy-4-(methylthio)butanoic acid;
35
               [0413]
                      2-formylamino-4-(methylthio)butanoic acid;
               [0414]
                      2-acetylamino-4-(methylthio)butanoic acid;
               [0415]
```

```
[0416]
                      2-propionylamino-4-(methylthio)butanoic acid;
              [0417]
                      2-butyrylamino-4-(methylthio)butanoic acid;
                      2-benzoylamino-4-(methylthio)butanoic acid;
              [0418]
              [0419]
                      2-lactoylamino-4-(methylthio)butanoic acid;
              [0420]
5
                      2-[2-carboxy-2-(hydroxy)propionylamino]-4-
         (methylthio) butanoic acid;
              [0421]
                      2-[2-carboxy-1-(hydroxy)propionylamino]-4-
         (methylthio) butanoic acid;
                      2-[2-carboxy-1,2-(dihydroxy)propionylamino]-4-
              [0422]
10
         (methylthio) butanoic acid;
              [0423]
                      2-[hydroxy(phenyl)acetyl]amino-4-
         (methylthio) butanoic acid;
              [0424]
                      2-[2,3-dicarboxy-2-(hydroxy)butyrylamino]-4-
         (methylthio) butanoic acid;
15
                      2-[2-carboxy-1-carboxymethyl-1-
              [0425]
         (hydroxy)propionylamino]-4-(methylthio)butanoic acid;
              [0426]
                      2-(3-carboxyacryloyl)amino-4-
         (methylthio) butanoic acid;
              [0427]
                      2-(2,4-pentadienoylamino)-4-
20
         (methylthio) butanoic acid;
                      2-(2-carboxypropionylamino)-4-
              [0428]
         (methylthio) butanoic acid;
              [0429]
                      2-[(4-carboxy)amylamino]-4-
         (methylthio) butanoic acid;
25
              [0430]
                      2-glycoloylamino-4-(methylthio) butanoic acid;
              [0431]
                      2-glutaroylamino-4-(methylthio)butanoic acid;
                      2-carboxy-4-(ethylthio)butanoic acid;
              [0432]
              [0433]
                      2-acetyloxy-4-(ethylthio)butanoic acid;
              [0434]
                      2-propionyloxy-4-(ethylthio)butanoic acid;
30
              [0435]
                      2-butyryloxy-4-(ethylthio)butanoic acid;
              [0436]
                      2-benzoyloxy-4-(ethylthio)butanoic acid;
                      2-lactoyloxy-4-(ethylthio)butanoic acid;
              [0437]
              [0438]
                      2-[2-carboxy-2-(hydroxy)propionyloxy]-4-
         (ethylthio) butanoic acid;
35
              [0439]
                      2-[2-carboxy-1-(hydroxy)propionyloxy]-4-
         (ethylthio) butanoic acid;
```

```
2-[2-carboxy-1,2-(dihydroxy)propionyloxy]-4-
              [0440]
         (ethylthio) butanoic acid;
              [0441]
                      2-[hydroxy(phenyl)acetyl]oxy-4-
         (ethylthio) butanoic acid;
                      2-[2,3-dicarboxy-2-(hydroxy)butyryloxy]-4-
              [0442]
5
         (ethylthio) butanoic acid;
              [0443]
                     2-[2-carboxy-1-carboxymethyl-1-
         (hydroxy)propionyloxy]-4-(ethylthio)butanoic acid;
                      2-(3-carboxyacryloyl)oxy-4-(ethylthio)butanoic
              [0444]
         acid:
10
              [0445] 2-(2,4-pentadienoyloxy)-4-(ethylthio)butanoic
         acid:
                      2-(2-carboxypropionyloxy)-4-
              [0446]
         (ethylthio) butanoic acid;
                      2-[(4-carboxy)amyloxy]-4-(ethylthio)butanoic
15
              [0447]
         acid;
                      2-glycoloyloxy-4-(ethylthio)butanoic acid;
              [0448]
                      2-glutaroyloxy-4-(ethylthio)butanoic acid;
              [0449]
                      2-formylamino-4-(ethylthio)butanoic acid;
              [0450]
                      2-acetylamino-4-(ethylthio)butanoic acid;
              [0451]
20
                      2-propionylamino-4-(ethylthio)butanoic acid;
              [0452]
                      2-butyrylamino-4-(ethylthio)butanoic acid;
              [0453]
                      2-benzoylamino-4-(ethylthio)butanoic acid;
              [0454]
                      2-lactoylamino-4-(ethylthio)butanoic acid;
              [0455]
                      2-[2-carboxy-2-(hydroxy)propionylamino]-4-
              [0456]
25
         (ethylthio) butanoic acid;
                      2-[2-carboxy-1-(hydroxy)propionylamino]-4-
               [0457]
         (ethylthio) butanoic acid;
                      2-[2-carboxy-1,2-(dihydroxy)propionylamino]-4-
               [0458]
         (ethylthio) butanoic acid;
30
                      2-[hydroxy(phenyl)acetyl]amino-4-
               [0459]
         (ethylthio) butanoic acid;
                      2-[2,3-dicarboxy-2-(hydroxy)butyrylamino]-4-
               [0460]
          (ethylthio) butanoic acid;
                      2-[2-carboxy-1-carboxymethyl-1-
               [0461]
35
          (hydroxy) propionylamino] -4- (ethylthio) butanoic acid;
```

```
[0462] 2-(3-carboxyacryloyl)amino-4-
         (ethylthio) butanoic acid;
               [0463]
                       2-(2,4-pentadienoylamino)-4-
         (ethylthio)butanoic acid;
 5
               [0464]
                       2-(2-carboxypropionylamino)-4-
         (ethylthio) butanoic acid;
               [0465]
                       2-[(4-carboxy)amylamino]-4-(ethylthio)butanoic
         acid:
                       2-glycoloylamino-4-(ethylthio)butanoic acid;
               [0466]
10
                       2-glutaroylamino-4-(ethylthio)butanoic acid;
               [0467]
               [0468]
                       2-carboxy-4-(propylthio) butanoic acid;
               [0469]
                       2-acetyloxy-4-(propylthio)butanoic acid;
               [0470]
                       2-propionyloxy-4-(propylthio)butanoic acid;
                       2-butyryloxy-4-(propylthio)butanoic acid;
               [0471]
15
               [0472]
                      2-benzoyloxy-4-(propylthio)butanoic acid;
               [0473]
                      2-lactoyloxy-4-(propylthio)butanoic acid;
               [0474]
                       2-[2-carboxy-2-(hydroxy)propionyloxy]-4-
         (propylthio) butanoic acid;
                       2-[2-carboxy-1-(hydroxy)propionyloxy]-4-
               [0475]
20
         (propylthio) butanoic acid;
                       2-[2-carboxy-1,2-(dihydroxy)propionyloxy]-4-
               [0476]
         (propylthio) butanoic acid;
               [0477]
                      2-[hydroxy(phenyl)acetyl]oxy-4-
         (propylthio) butanoic acid;
25
                      2-[2,3-dicarboxy-2-(hydroxy)butyryloxy]-4-
               [0478]
         (propylthio) butanoic acid;
              [0479]
                      2-[2-carboxy-1-carboxymethyl-1-
         (hydroxy)propionyloxy]-4-(propylthio)butanoic acid;
              [0480]
                      2-(3-carboxyacryloyl)oxy-4-
30
         (propylthio) butanoic acid;
              [0481]
                      2-(2,4-pentadienoyloxy)-4-(propylthio)butanoic
         acid;
              [0482]
                      2-(2-carboxypropionyloxy)-4-
         (propylthio) butanoic acid;
35
              [0483]
                      2-[(4-carboxy)amyloxy]-4-(propylthio)butanoic
         acid:
              [0484]
                      2-glycoloyloxy-4-(propylthio)butanoic acid;
```

```
2-glutaroyloxy-4-(propylthio)butanoic acid;
              [0485]
              [0486]
                      2-formylamino-4-(propylthio)butanoic acid;
                      2-acetylamino-4-(propylthio)butanoic acid;
              [0487]
              [0488]
                      2-propionylamino-4-(propylthio)butanoic acid;
                      2-butyrylamino-4-(propylthio)butanoic acid;
5
              [0489]
                      2-benzoylamino-4-(propylthio)butanoic acid;
              [0490]
                      2-lactoylamino-4-(propylthio)butanoic acid;
              [0491]
                      2-[2-carboxy-2-(hydroxy)propionylamino]-4-
              [0492]
         (propylthio) butanoic acid;
              [0493]
                      2-[2-carboxy-1-(hydroxy)propionylamino]-4-
10
         (propylthio) butanoic acid;
              [0494]
                      2-[2-carboxy-1,2-(dihydroxy)propionylamino]-4-
         (propylthio) butanoic acid;
                      2-[hydroxy(phenyl)acetyl]amino-4-
              [0495]
         (propylthio) butanoic acid;
15
                      2-[2,3-dicarboxy-2-(hydroxy)butyrylamino]-4-
              [0496]
         (propylthio) butanoic acid;
                      2-[2-carboxy-1-carboxymethyl-1-
              [0497]
         (hydroxy) propionylamino] -4- (propylthio) butanoic acid;
                      2-(3-carboxyacryloyl)amino-4-
20
         (propylthio) butanoic acid;
              [0499]
                      2-(2,4-pentadienoylamino)-4-
         (propylthio) butanoic acid;
              [0500]
                      2-(2-carboxypropionylamino)-4-
         (propylthio) butanoic acid;
25
                      2-[(4-carboxy)amylamino]-4-
              [0501]
         (propylthio) butanoic acid;
                      2-glycoloylamino-4-(propylthio)butanoic acid;
              [0502]
                      2-glutaroylamino-4-(propylthio)butanoic acid;
              [0503]
              [0504]
30
              [0505]
                      2-carboxy-4-(butylthio)butanoic acid;
                      2-acetyloxy-4-(butylthio)butanoic acid;
              [0506]
              [0507]
                      2-propionyloxy-4-(butylthio)butanoic acid;
                      2-butyryloxy-4-(butylthio)butanoic acid;
              [0508]
              [0509]
                      2-benzoyloxy-4-(butylthio)butanoic acid;
35
                      2-lactoyloxy-4-(butylthio)butanoic acid;
              [0510]
```

```
2-[2-carboxy-2-(hydroxy)propionyloxy]-4-
         (butylthio) butanoic acid;
                      2-[2-carboxy-1-(hydroxy)propionyloxy]-4-
              [0512]
         (butylthio) butanoic acid;
              [0513]
                      2-[2-carboxy-1,2-(dihydroxy)propionyloxy]-4-
5
         (butylthio) butanoic acid;
                      2-[hydroxy(phenyl)acetyl]oxy-4-
              [0514]
         (butylthio) butanoic acid;
                      2-[2,3-dicarboxy-2-(hydroxy)butyryloxy]-4-
              [0515]
         (butylthio) butanoic acid;
10
                      2-[2-carboxy-1-carboxymethyl-1-
              [0516]
         (hydroxy) propionyloxy] -4- (butylthio) butanoic acid;
              [0517] 2-(3-carboxyacryloyl)oxy-4-(butylthio)butanoic
         acid;
              [0518] 2-(2,4-pentadienoyloxy)-4-(butylthio)butanoic
15
         acid;
              [0519] 2-(2-carboxypropionyloxy)-4-
         (butylthio) butanoic acid;
                      2-[(4-carboxy)amyloxy]-4-(butylthio)butanoic
              [0520]
         acid;
20
                      2-qlycoloyloxy-4-(butylthio)butanoic acid;
              [0521]
                      2-glutaroyloxy-4-(butylthio)butanoic acid;
              [0522]
                      2-formylamino-4-(butylthio)butanoic acid;
              [0523]
                      2-acetylamino-4-(butylthio)butanoic acid;
              [0524]
              [0525]
                      2-propionylamino-4-(butylthio)butanoic acid;
25
                      2-butyrylamino-4-(butylthio)butanoic acid;
              [0526]
              [0527]
                      2-benzoylamino-4-(butylthio)butanoic acid;
                      2-lactoylamino-4-(butylthio)butanoic acid;
              [0528]
                      2-[2-carboxy-2-(hydroxy)propionylamino]-4-
              [0529]
         (butylthio) butanoic acid;
30
                      2-[2-carboxy-1-(hydroxy)propionylamino]-4-
               [0530]
         (butylthio) butanoic acid;
                      2-[2-carboxy-1,2-(dihydroxy)propionylamino]-4-
               [0531]
         (butylthio) butanoic acid;
                      2-[hydroxy(phenyl)acetyl]amino-4-
               [0532]
35
         (butylthio) butanoic acid;
```

```
(butylthio)butanoic acid;
                                                                                                                                                                                                             2-[2,3-dicarboxy-2-(hydroxy)butyrylamino]-4-
                                                                                                                                                                           10534] 2-12-carboxy-1-carboxymethy1-1-
                                                                                                                                                     (hydroxy) propionylaminol -4 - (butylthio) butanoic acid;
                                                                                                                     5
                                                                                                                                              (butylthio)butanoic acid;
                                                                                                                                                            [0536] 2-(2,4-pentadienoylamino)-4-
                                                                                                                                       (butylthio)butanoic acid;
                                                                                               10
                                                                                                                                                    [0537] 2-(2-carboxypropionylamino)-4-
                                                                                                                               (butylthio)butanoic acid;
                                                                                                                        acid;
                                                                                                                                                                       2-[(4-carboxy)amylamino]-4-(butylthio)butanoic
                                                                                                                                    1, [10539] 2-91ycoloylamino-4-(butylthio)butanoic acid;
                                                                            15
                                                                                                                 a_{IQ}
                                                                                                                               [0540]
                                                                                                  of Formula I is selected from the group of compounds

'a marhy,' n 'a o' the group of compounds

'a hindrow,' the compounds
                                                                                                                                                       2-glutaroylamino-4-(butylthio)butanoic acid.
                                                                                              wherein R1 is selected from the group of compounds of formic acid. Scatic acid. Sca
                                                                                          wherein Ri is methyl; n is 2; Re is hydroxy or occor; acid, propionic acid, malic acid
                                                         20
                                                                                       acid, butyric acid, benzoic acid, acetic acid, mannalic acid, lactic acid, malic acid, malic acid, malic acid, acid, malic acid,
                                                                                   acid, butyric acid, benzoic acid, lactic acid, malic acid, horic acid, citric acid, fumaric acid, acid, acid, acid, acid, acid, fumaric acid, acid,
                                                                               Sorbic acid, mandelic acid, citric acid, tumaric acid, acid, adipic acid, acid
                                                                           SOTDIC acid, boric acid, succinic acid, adipic acid, or glutaric acid. In an even more preferred from the
                                                                        Slycolic acid, or slutaric acid. In an even more preferred from the
                                      25
                                                                   embodiment, the compound of Formula I is selected from derivative of formic acic
                                                               group of compounds wherein Ri is methyl; n is 2; Ri is nitric acid, or ricric acid,
                                                           propionic acid, butyric acid, lactic acid, citric acid, or
                  30
                                                                        10542]

Representative salts of the compound of
                                                Formula I include the ammonium, magnesium, calcium, calcium,
                                             Iithium, sodium, the ammonium, magnesium, calcium, rn a nreferred embodiment the commonium, the compet, and
                                         Sinc salts. In a preferred embodiment, the compound of
                                     Formula I is in the form of the calcium salt.
                                  Representative amides include methylamide, dimethylamide,
35
                              ethylmethylamide, butylamide, dibutylamide, dibutylamide,
                          ethylmethylamide, butylamide, dibutylamide, butylamide, alkyl ester of N-acyl methionates (e.g., normalise, inclinde)
                      alkyl N-acetyl methionates. Representative esters include
```

the methyl, ethyl, n-propyl, isopropyl, butyl esters, namely n-butyl, sec-butyl, isobutyl, and t-butyl esters, pentyl esters and hexyl esters, especially n-pentyl, isopentyl, n-hexyl and isohexyl esters.

5

10

15

20

25

30

35

[0543] In various preferred embodiment, the compound of Formula I is 2-hydroxy-4-(methylthio) butanoic acid (HMBA) or a salt, amide or ester thereof. In still more preferred embodiments, the compound of Formula I is HMBA.

[0544] Preferably, the concentration of the compound of Formula I in the feed compositions described herein is between about 0.01% and about 5%. In various preferred embodiments, the concentration is between 0.01% and about 4%; between 0.02% and about 3%; between 0.03% and about 2%; between 0.04% and about 1%; between about 0.05% and about 0.6%; and between about 0.06% and about 0.525%. In various particularly preferred embodiments, the concentration is about 0.075%; about 0.125%; about 0.15%; about 0.225%; about 0.25%; about 0.3%; about 0.375%; and about 0.5%.

In another embodiment of the present invention, the methods of inhibiting microbes in animal feed comprises treating said feed with a compound of Formula I and one or more organic acids. Preferably, the organic acid has a pK<sub>a</sub> < 5.5. In one embodiment, the organic acid is a carboxyl-substituted hydrocarbon moiety. The hydrocarbon moiety may be further substituted by one or more substituents such as halogen; oxygen-containing groups such as alkoxy, aryloxy, hydroxy, protected hydroxy, keto, acyl, acyloxy; nitrogen-containing groups such as nitro, amino, amido, cyano; and sulfur-containing groups such as thiol, thioalkyl, and sulfonyl. In a preferred embodiment, said organic acids are selected from the group consisting of formic acid, acetic acid, propionic acid, butyric acid, benzoic acid, lactic acid, malic acid, tartaric acid, mandelic acid, citric acid, fumaric acid, sorbic acid, boric acid, succinic acid, adipic acid, glycolic acid, and glutaric acid, or combinations thereof. In one embodiment,

the organic acid is formic acid, propionic acid, butyric acid, lactic acid, or combinations thereof.

5

10

15

20

25

30

35

Preferably, the combined concentration of the compound of Formula I and the organic acid or mixture of organic acids in the food compositions described herein is between about 0.01% and about 5%. In various preferred embodiments, the combined concentration is between about 0.015% and about 4%; between about 0.02% and about 3%; between about 0.05% and about 2.5%; between about 0.075% and about 2%; between about 0.1% and about 1.5%; between about 0.15% and about 1%; between about 0.4% and about 0.9%; between about 0.5% and about 0.8%; between about 0.01% and about 5%; between about 0.01% and about 4.5%; between about 0.05% and about 4%; between about 0.08% and about 3%; between about 0.1% and about 2.5%; between about 0.01% and about 0.8%; between about 0.01% and about 0.5%; between about 0.05% and about 0.6%; and between about 0.06% and about 0.525%.

In various other preferred embodiments, the concentration of said compound of Formula I and said organic acid in the food compositions described herein is as follows:

Concentration of the compound of Formula I	Concentration of the organic acid
between about 0.01% and about 0.5%	between about 0.01% and about 0.5%
between about 0.1% and about 0.4%	between about 0.1% and about 0.5%
about 0.125%	about 0.375%
about 0.225%	about 0.225%
about 0.25%	about 0.25%
about 0.375%	about 0.125%
about 0.3%	about 0.5%

[0547] In a preferred embodiment of the present invention, the antimicrobial compositions comprises a

compound of Formula I and one or more other acidulants. Such acidulants are typically strong acids, and are preferably mineral acids. Examples of such acidulants include phosphoric acid, phosphorous acid, sulfuric acid, hydrochloric acid, hydrobromic acid, and nitric acid. In one embodiment, the acidulant is phosphoric acid.

5

10

15

20

25

30

35

[0548] In a more preferred embodiment, the antimicrobial compositions comprise a compound of Formula I, one or more organic acids, as defined above, and one or more other acidulants as defined above.

[0549] Preferably, the pH of the feed is between about 3 and about 8. Even more preferably, the pH is between about 4 and about 7. Still more preferably, the pH is between about 4.5 and about 6.75. The pH may be measured by placing a known quantity of the feed and placing it in a known quantity of distilled water. The pH of the resulting solution, after sitting, may be measured by any standard means for measuring pH.

[0550] The following embodiments are particularly preferred for the addition of combinations of Alimet® and formic acid to feed (concentrations expressed in wt% of feed composition):

[0551] about 0.125% Alimet® and about 0.375% formic acid at pH from about 4.5 to about 6.75;

[0552] about 0.25% Alimet® and about 0.25% formic acid at pH from about 4.5 to about 6.75;

[0553] about 0.375% Alimet® and about 0.125% formic acid at pH from about 4.5 to about 6.75;

[0554] about 0.5% Alimet® at pH from about 4.5 to about 6.75;

[0555] about 0.3% Alimet® and about 0.5% formic acid at pH from about 4.5 to about 6.75.

[0556] In another preferred embodiment, the abovementioned organic acid is a mixture of formic acid and propionic acid, wherein the formic acid comprises from about 95% to about 5% of the organic acid mixture and the propionic acid comprises from about 5% to about 95% of the organic mixture. Preferably, formic acid comprises from about 85% to about 15% of the organic acid mixture, and propionic acid comprises from about 15% to about 85% of the organic acid mixture. In another preferred embodiment, formic acid comprises from about 85% to about 65% of the organic acid mixture, and propionic acid comprises from about 15% to 35% of the organic acid mixture. In another preferred embodiment, formic acid comprises about 75% of the organic acid mixture, and propionic acid comprises about 25% of the organic acid mixture. This formic/propionic acid mixture can then be combined with the compound of Formula I according to the ratios described above.

5

10

15

20

25

30

35

In a preferred embodiment, the antimicrobial compositions comprise a compound of Formula I, preferably HMBA or a salt thereof, and a first organic acid, as defined herein. Preferably, the first organic acid is selected from the group consisting of formic acid, acetic acid, propionic acid, butyric acid, benzoic acid, lactic acid, malic acid, tartaric acid, mandelic acid, citric acid, fumaric acid, sorbic acid, boric acid, succinic acid, adipic acid, glycolic acid, and glutaric acid. In another preferred embodiment, the antimicrobial compositions may further comprise one or more components selected from: a second organic acid, a third organic acid, and an acidulant. Preferably, the second organic acid and third organic acid are independently selected from the group consisting of formic acid, acetic acid, propionic acid, butyric acid, benzoic acid, lactic acid, malic acid, tartaric acid, mandelic acid, citric acid, fumaric acid, sorbic acid, boric acid, succinic acid, adipic acid, glycolic acid, and glutaric acid. Preferably, the acidulant is selected from the group consisting of phosphoric acid, sulfuric acid, phosphorous acid, hydrochloric acid, hydrobromic acid, and nitric acid.

[0558] It has been discovered that certain antimicrobial compositions of the invention have an

improved odor, when the compound of Formula I is Alimet<sup>®</sup>, compared to similar compositions without Alimet<sup>®</sup>. For example, blends comprising formic acid have a pungent odor. In such blends without Alimet<sup>®</sup>, this odor is more readily detectable than in the same blends containing Alimet<sup>®</sup>. Without being limited to a particular theory, it is believed that the Alimet<sup>®</sup> in the blends may lower the vapor pressure of the other organic acids in the blends. Alternatively, the Alimet<sup>®</sup> may mask the disagreeable odors.

[0559] In another preferred embodiment is provided a method for inhibiting bacteria in silage, said method comprising treating said silage with an anti-bacterial composition comprising a compound of Formula I. Preferably, the compound of Formula I is added to the silage at about 1 lb/ton to 80 lb/ton of fresh forage, more preferably at about 2 lb/ton to 50 lb/ton of fresh forage, more preferably about 3 lb/ton to 45 lb/ton of fresh forage, more preferably about 4 lb/ton to 40 lb/ton of fresh forage, more preferably about 5 lb/ton to 35 lb/ton of fresh forage, more preferably about 7 lb/ton to 30 lb/ton of fresh forage, more preferably about 9 lb/ton to 25 lb/ton of fresh forage, more preferably about 9 lb/ton to 25 lb/ton of fresh forage, more preferably about 10 lb/ton to 20 lb/ton of fresh forage. Optionally, the compositions may further comprise an acidulant, as described herein.

[0560] In another preferred embodiment is provided a method for inhibiting bacteria in silage, said method comprising treating said silage with an anti-bacterial composition comprising a compound of Formula I and one or more organic acids as described above. Preferably, the compound of Formula I and other organic acid(s) are added to the silage at about 2 lb/ton to 125 lb/ton of fresh forage combined. In one embodiment, the compound of Formula I and other organic acid(s) are added to the silage at about 4 lb/ton to 100 lb/ton of fresh forage combined. In another embodiment, the compound of Formula I and other organic acid(s) are added to the silage at about 5 lb/ton to 90 lb/ton of fresh forage combined, more preferably

about 7 lb/ton to 80 lb/ton of fresh forage combined, more preferably about 8 lb/ton to 70 lb/ton of fresh forage combined, more preferably about 9 lb/ton to 60 lb/ton of fresh forage combined, more preferably about 10 lb/ton to 55 lb/ton of fresh forage combined, more preferably about 12 lb/ton to 50 lb/ton of fresh forage combined, more preferably about 15 lb/ton to 30 lb/ton of fresh forage combined. Optionally, the compositions may further comprise an acidulant, as described herein.

10

15

5

[0561] In a preferred embodiment of the invention, the bacteria inhibited according to the methods of the present invention is from the family Enterobacteriaceae, Campylobacter or Lactobacillaceae. In another preferred embodiment, the bacteria is from the family Campylobacter or Lactobacillaceae. In another preferred embodiment, the bacteria is from the genus Lactobacillus or Campylobacter. In another preferred embodiment, the bacteria is L. plantarum or C. jejuni. In a particularly preferred embodiment, the bacteria is from the family Enterobacteriaceae. In an even more preferred embodiment, the bacteria is from the genus Salmonella or Escherichia. In a still more preferred embodiment, the bacteria is S. enteritidis or E. coli.

20

[0562] In yet another embodiment of the present invention, the above-mentioned compounds of Formula I may be used in the manufacture of a nutrient composition for inhibiting bacteria in animal feed. These nutrient compositions may further comprise one or more organic acids, as described above. Optionally, the compositions may

further comprise an acidulant, as described herein.

30

25

[0563] In still yet another embodiment of the present invention, the above-mentioned compounds of Formula I may be used in a method for inhibiting bacteria in animal feed comprising computing the concentration in said feed of said compound of Formula I necessary to inhibit bacteria present in said animal feed, and applying said compound of Formula

I to said feed in an amount sufficient to achieve said concentration.

5

10

15

20

25

30

35

[0564] In another embodiment of the present invention, the above-mentioned compounds of Formula I and above-mentioned organic acids may be used in a method of inhibiting bacteria in animal feed comprising computing the concentration in said feed of a compound of Formula I and another organic acid or mixture of organic acids necessary to inhibit bacteria present in said animal feed, and applying said compound of Formula I and said organic acid or mixture of organic acids to said feed in an amount sufficient to achieve said concentration.

[0565] In still yet another embodiment of the present invention, the above-mentioned compounds of Formula I may be used in a method of method for inhibiting mold in animal feed comprising directly or indirectly making information available for computing the concentration in said feed of a compound of Formula I necessary to inhibit bacteria present in said animal feed, and directly or indirectly making information available for applying said compound of Formula I to said feed in an amount sufficient to achieve said concentration.

[0566] In another embodiment of the present invention, the above-mentioned compounds of Formula I and above-mentioned organic acids may be used in a method of method for inhibiting mold in animal feed comprising directly or indirectly making information available for computing the concentration in said feed of said compound of Formula I and said organic acid or mixture of organic acids necessary to inhibit bacteria present in said animal feed, and directly or indirectly making information available for applying said compound of Formula I and said organic acid or mixture of organic acids to said feed in an amount sufficient to achieve said concentration.

[0567] In another embodiment of the present invention, the above-mentioned animal feeds may be heat-treated, either before or after administration of the

above-mentioned compounds of Formula I and/or organic acids.

5

10

15

20

25

30

35

[0568] In yet another embodiment of the present invention, the above-mentioned compounds of Formula I may be used in a method of inhibiting bacteria in animal feed, the method comprising monitoring the concentration of methionine supplement in said feed composition, adding additional amounts of said methionine supplement as needed to achieve an anti-bacterially effective concentration of methionine supplement in said feed composition.

Treatment of the animal feed compositions with [0569] the compounds of Formula I and with the other organic acids disclosed herein may be done by mixing the compound of Formula I (and other organic acid, if present) with the other ingredients in the feed, such as the corn, soybean meal, meat meal premix, other feed supplements, etc., as the feed is being formulated. Alternatively, the compound of Formula I and optional other organic acid(s) may be applied to a pre-mixed or pre-pelleted feed. In either case, the compound of Formula I and optional organic acid(s) are preferably added as liquids, and uniformly disperse throughout the bulk of the feed composition when applied. When the compound of Formula I and another organic acid are both used in the methods of the present invention, preferably said compound of Formula I and said other organic acid or acids are mixed together before application to the animal feeds. This pre-mixed compound of Formula I/organic acid(s) blend can be applied to the animal feed ingredients during formulation of the feed compositions, or can be applied to pre-mixed or pre-pelleted feed.

[0570] In one embodiment of the present invention is presented a method of inhibiting mold in an animal feed composition, the method comprising applying a compound of Formula I to said feed composition, wherein said feed composition comprises corn and soy.

[0571] In another embodiment of the present invention, the methods of inhibiting mold in animal feed

NVI 5252.4 PATENT

comprises treating said feed with an antifungally-effective comprises treating said feed with an antitungally-effective organic amount of a compound arrive as described above. Preferred embodiment; the above105721
105721 [0572] In another preferred embodiment, acid and from in acid is a mixture of formic acid from mentioned organic acid the formic acid commercians acid wherein the formic acid commercians mentioned organic acid is a mixture of formic acid and the formic acid comprises from the formic acid comprises and the formic acid comprises and the formic acid mixture and the formic acid and mixture of formic acid and mixture of formic acid and the formic acid and the formic acid and the formic acid comprises from the formic acid comprises and the for propionic acid, wherein the formic acid comprises from the and the organic acid mixture and the propionic acid, wherein the organic se to shout of the organic se to shout of the about 95% to about 5% of from shout se to shout organic acid comprises from shout se to shout of the organic acid comprises from shout se to shout of the organic acid comprises from shout of the organic acid comprises to shout of the organic acid comprises the organic acid mixture and the organic acid mixture acid. about 95% to about 5% of the organic acid mixture and the from about 5% to about 95% from about 5% to about 95% from propionic acid comprises from formic acid mixture and the the acid mixture and the acid mixture and the acid mixture and the acid mixture and the acid mixture acid mi acids as described above. proplonic acid comprises from about 5% to about 95% of the formic acid comprises from formic acid comprises and formic acid mixture and organic mixture. 15% of the organic mixture about 15% of the organic mixture about 15% of the organic mixture. organic mixture. Preterably, tormic acid mixture, and organic acid mixture are of the organic acid mixture are of the about 15% of from about 15% to about about 25% of from about 25% to about acid comprises from about 25% to acid comprises from acid comprises fr about 85% to about 15% of the organic acid mixture, and the about 15% to about 15% to about 15% to about 15% to ambout 15% to am proplonic acid mixture. From about 15% to about 85% of corganic acid mixture. organic acid mixture. In another preferred embodiment, the from about 85% to about 65% of from about 85% to about 65% of from formic acid comprises and provious acid mixture acid mixture and provious acid mixture acid mixture and provious acid mixture formic acid comprises from about 85% to about 65% of the order order organic acid mixture, and propionic acid mixture organic acid mixture, arranic acid m organic acid mixture, and propionic acid mixture. This organic acid mixture and propionic acid mixture. The communal of the organic acid mixture communal of the organic acid mixture acid mixture are then he combined with the communal of about 15% to 35% of the combined with the communal organic acid mixture can then he combined with the communal organic acid mixture can then he combined with the communal organic acid mixture can then he combined with the common organic acid mixture can then he combined with the common organic acid mixture. about 15% to 35% of the organic acid mixture. This organic acid mixture can then be combined with the compound of acid mixture can then to the ration described above acid mixture can then to the ration described acid mixture can then to the ration described above the compound of the combined with the compound of acid mixture can then to the ration described acid mixture can then to the ration described acid mixture can then to the ration described acid mixture can then the ration described acid mixture. la l according to the ratios described above. invention feed the present invention mold in an animal feed [10573] In another embodiment of inhihiting mold in an animal feed in the present and in an animal feed in the present animal feed acia mixture can then be combined with the compound

the ratios described above.

Formula I according to the ration of the ratio 105731 In another embodiment of the present invention of the present invention and in an animal feed inhibiting mold in an animal of inhibiting anniving a common of inhibiting anniving a common of the presented a method of inhibiting anniving a common of the present invention and the present invention of the p 18 Presented a method comprising applying a compound of composition, composition, and one or more organic acids to said feed 20 composition the method comprising applying a compound to said feed to said feed composition commisses formula I and one or more feed commosition commisses formula I and wherein eaid feed commosition Formula I and one or more organic acids to said feed corn

rormula I and one or more organic acids to comprises corn

said feed composition acid is said feed composition.

composition, wherein said said organic acid is said feed corn

and any one amandiment acid feed composition acid is said feed corn

and any one amandiment acid feed composition acid is said feed corn

and any one amandiment acid feed composition acid is said feed corn

composition, one amandiment acid feed composition acid is said feed corn

composition, one amandiment acid feed composition acid is said feed composition acid is said feed composition. composition, wherein said teed composition acid is selected said organic acid is selected.

In one embodiment, formic acid aretic acid and soy. In one consisting of formic acid aretic acid aretic acid. and soy. In one embodiment, said organic acid, lactic acid, the group consisting acid, henroic acid, henroic acid.

from the group hurvric acid. from the group consisting of formic acid, lactic acid, benzoic acid, citric acid.

propionic acid, butyric acid, mandelic acid, citric 15 propionic acid, butyric acid, mandelic acid, citric acid, mandelic malic acid, tartaric acid, mandelic acid, succinic acid, boric acid, succinic acid, succinic acid, sorbic acid, and cluraric acid, and cluraric acid, always acid, and cluraric acid, acid, acid, and cluraric acid, acid, and cluraric acid, ac adipic acid, thereof. In another embodiment, acid or combinations thereof. arid or combinations combinations thereof. tumaric acid, sorpic acid, and glutaric acid, the rect acid, and glutaric acid, acid, and glutaric acid, the rect acid, and glutaric acid, the rect acid, and glutaric acid, acid, and glutaric acid, the rect acid, and glutaric acid, acid, and glutaric acid, acid, and glutaric acid, acid, and glutaric acid, combinations thereof.

In another embodiment, the organ another embodiment, the organ propionic acid, or combinations acid, or combinations acid is formic acid, propionic acid. 20 OI. In yet another embodiment of the present invention:

in a markon for delaying the present may

invention:

in a markon for delaying the formation of more marked the markon for delaying the ma invention, the above-mentioned compounds of Formula I may the formation of mold in the formation, the above-mentioned the formation are arrived to the method commercial or be used in a method for delaying the rormation of mold in wherein an animal feed composition, said feed composition as a composition of Formila T to said feed composition. an animal feed composition, the method composition, wherein a compound of Formula I to said feed corn and any a compound of feed common common a compound of feed common c 25 said feed composition comprises corn and soy. thereof. 30 35

[0575] In yet another embodiment of the present invention. invention, the above-mentioned compounds or Formula 1 and for a method for a above-mentioned may be used in a method for above-mentioned organic acids may be used in an animal feed above-mention of mold in a method for animal feed animal feed and a method for an animal feed animal feed animal feed animal feed and feed animal feed and feed animal feed a delaying the tormation of more organic acide to eaid feed

composition, the method comprising applying a caid feed

composition, the method organic acide to eaid feed above-mentioned organic acids may be used in a mer animal feed in an animal feed in a morning of mold in an animal feed delaying the formation of morning or morning composition, the method comprising applying a compound composition, the method comprising acids to said feed acids to said feed acids to said feed composition and one or more organic acids to comprising applying a compound feed acids to said feed composition composition. Formula I and one or more organic acids to said feed composition comprises corn composition, wherein said feed composition, and any wherein said feed composition. invention:

In a method of inhihiting the formation of more inhihiting the method of inhihiting the inged in a method of inhibiting the inged in a method of inhihiting the inged in a method of inhihiting the inged in a method of inhihiting the index in a method of inhihiting the inhihiting the index in a method of inhihiting the inhihiti invention, the above-mentioned compounds of Formula I may in the above-mentioned compounds of Formula I may in a method of inhibiting the method comprision and in a method of the method comprision the method compounds of Formula I may in a more in the method compounds of Formula I may in the more in the method compounds of Formula I may in the more in the method compounds of Formula I may in the more in the method compounds of Formula I may in the more in the more in the method compounds of Formula I may in the more in the more in the method compounds of Formula I may in the more in the more in the method compounds of Formula I may in the more in the more in the method of interest in the method compounds of Formula I may in the more in the more in the more in the more in the method of the more in the method of the more in the method compounds in the method c be used in a method of inhibiting the method comprising wherein an animal feed composition, asid feed composition, asid feed composition, asid feed composition. an animal feed composition, the method comprising applying the method composition, wherein a said feed composition, about 17% of a modern a compound of Formula I to said feed content of abut 17% or nontent is at least noise and feed composition has a moisture content is at least noise said feed composition has a moisture content is at least noise said feed composition has a moisture content is at least noise said feed composition has a moisture content is at least noise said feed composition. said feed composition has a moisture content of abut 0.01%.

the moisture content is at least le less. Preterably:

the moisture content is at least 1 5 In another embodiment, the moisture content is at least 1%.

In another embodiment, the moisture content is at least 1.

In another embodiment, the moisture content is at least 1.

In another embodiment. and soy. In another embodiment, the moisture content is at least in another embodiment, the moisture content is at least in another embodiment, the moisture content is at least. invention, the above-mentioned compounds of rormula inventioned or arganic acide may be used in a method of above-mentioned organic acide may be used in a method of above-mentioned organic acide may be used in a method of above-mentioned organic acide may be used in a method of above-mentioned organic acide may be used in a method of acide may be used in a meth 10 invention, the above-mentioned compounds of Formula I and the above-mentioned may be used in a method of above-mentioned formation of mold in an animal feed above-mentioned formation of mold in a method of the second feed above-mentioned formation of mold in an animal feed above-mentioned formation of mold in animal feed above-mentioned above-mentioned organic acids may be used in a method and in an animal feed inhibiting the formation account of a common of mold in an animal account inhibiting the mathod account of a common of the mathod account of the inhibiting the tormation of mold in an animal reed

inhibiting the method comprising applying a aid feed

composition, the method or more organic acide to aaid feed

composition, and one or more organic acide to a aid feed composition, the method comprising applying a compound to said feed acids to said feed formula I and one or more formula I and wherein said feed composition has a major formula it in the said feed composition has a major formula it in the said feed composition has a compound to said feed composition has a compound to said feed composition has a composition has a composition has a composition has a composite feed composition of the composition has a composite feed composition has a composite feed composite feed composition feed composition has a composite feed composite feed composition feed composite feed composit Formula I and one or more organic acids to said teed the moisture the 15 Composition, wherein said teed composition has a moisture preferably, the moisture preferably, the moisture of about 17% or less. 10%. content of about 17% or less. Preterably, the molsture ambodiment, the content is at least 0.01%. In another ambodiment content is at least 0.12° or 12° or content is at least 18 at least 18 Tr another embodiment.

The moisture content is at least 18 Tr another embodiment.

The another embodiment. molsture content is at least 10% In another embodiment, the moisture content is at least 10% In another embodiment, the moisture content is at least 10% 20 ure content 18 at least 10%.

The present of the pr invention is provided a method comprising treating early method comprising early method comprising treating early method comprising early method ea Invention is provided a method for innibiting mold in treating said silage with an comprising treating said silage of Formula T silage, said method comprising common of Formula and silage, said method comprising common of Formula and silage, said method comprising common of Formula and silage, said method comprising and common of Formula and silage, said method comprising and silage, said method comprising and silage. silage, said method comprising treating said silage with an incomposition comprising a compound of the added to the composition of Formula Tip added to the composition of Tip added to the co moisture content is at least 10%. 25 anti-tungal composition comprising a compound of formula is added to the compound of formula is added for an intron of freeh for an intro Preterably, the compound to to the silage at about 1 lb/ton to 40 lb/ton of fresh forage, more 30 35

preferably about a hour of the proferance of the profession of the preferably about 5 iblton to 30 iblton of tresh fresh to 30 iblton of inline preferably about 7 iblton to 25 iblton on inline preferably about 7 iblton in inline to 20 iblton in inline for and in inline preferably about 7 iblton in inline to 20 iblton inline to 20 iblton in inline to 20 iblton in inline to 20 iblton inline to 20 iblton in inline to 20 iblton i more preferably about 7 lb/ton to 25 lb/ton to 20 lb/ton of forage forage forage Torage. In yet another embodiment of the present invention is provided a method for treating early method a method for treating early method a method treating treating early method and treating treating early method and a method and treating treating early method and and treating treating early method and and treating treating early method and and treating treating treating early method and and treating treat invention is provided a method tor innibiting moid in with an treating said silage with a I silage, said method comprising a common of rormula I silage, said method comprising a common of sai silage, said method comprising treating said silage with an I graphical silage with an I graphical solution comprising a compound of the commound of preferance and composition acids anti-fungal composition acids acids anti-fungal composition acids acid anti-tungal composition comprising a compound of the compound and one or more organic acids. Preterably, the compound of the silage and one or more organic acid(s) are added to more organic acid(s) freeh for are nombined formula I and other organic acid(s) are about a latter to so introduce organic acid(s). Formula I and other organic acid(s) are added to the silar

Formula I and other organic acid(s) fresh for an intron of fresh for an intron of fresh for at about 5 lb/ton to 30 lh/ton to 40 lh/ton of fresh for at about 5 lb/ton ahour a lh/ton to 40 lh/ton to 40 lh/ton to 40 lh/ton of fresh for an about 5 lb/ton to 40 lh/ton to 40 lh/ton to 40 lh/ton of fresh for an about 5 lb/ton to 40 lh/ton of fresh for an about 5 lb/ton to 40 lh/ton to 40 lh fresh forage. at about 5 lb/ton to 50 lb/ton to 40 lb/ton to 30 lb/ton to 30 lb/ton to 40 lb/ton to 30 lb/ton more preterably about 8 lb/ton to 40 lb/ton to 30 lb/ton to 10 lb/ton to 30 lb/ton to 10 lb/ton to 30 lb/ton to 10 lb/ton to 30 lb/ton combined, more preferably about 10 lb/ton to about 15 lb/ton to fresh forage combined, nombined fresh forage fresh forage combined for a combined for 5 [0580] In yet another embodiment of the present may in a marked for inhihiting the growth of mold in the above-mentioned compounds of for inhihiting the growth of mold in a method for inhihiting the growth of mold in a method for inhihiting the growth of mold in a method for inhihiting the growth of mold in a method for inhihiting the growth of mold in a method for inhihiting the growth of mold in a method for inhihiting the growth of mold in a method for inhihiting the growth of mold in a method in a met invention, the above-mentioned compounds of Formula I may an the above-mentioned compounds the growth of mold in a method for inhibiting commortains committee the method committ 25 1b/ton of fresh forage combined. be used in a method for inhibiting the growth of mold in a feed composition, the method comparish of rorming animal feed composition in said feed of a composition in the method feed of a composition in the concentration in said feed of a composition in the concentration in the conc animal reed composition, the method comprising computing I the method of a compound of Formula I the concentration in the arouth of mold in easily feed the concentration in the arouth of mold in easily formula in the concentration in the arouth of mold in easily formula I the concentration in the arouth of mold in easily formula I animal reed composition. 10 the concentration in said feed of a compound of Formula from the growth of mold in said feed necessary to inhibit the growth common of sommer and a second common of sommer and a second common of sommer and second common of s necessary to innibit the growth of mold in said feed to recessary to innibit the growth of compound of formula I to and applying said concentration composition; and applying said concentration reed composition in said concentration.

The said concentration of the present of invention, anide may he weed in a method for inhihiting the composition; and applying said concentration.

said feed composition in said concentration. 15 Invention, the above-mentioned compounds of Formula I and the method for inhibiting the method for the method for the method organic acids may be used in feed common for the method organic acids may be animal feed common feed common for mold in an animal feed common feed common for mold in an animal feed common feed common feed common for mold in an animal feed common feed common for mold in an animal feed common feed common feed compounds of the method for the method common feed compounds of the method for inhibiting the method for inhibiting the method common feed compounds of the method for inhibiting the method for inhibiting the method common feed compounds of the method for inhibiting the method for inhib organic acids may be used in a method for inhibiting the method for method fo growth or more computing the concentration in said or mirror comprising computing tand another organic acid or mirror comprising companies to another organic acid or mirror companies to another organic acid or mirror companies to another organic acid or mirror organic acid or comprising computing the concentration in said feed of a mixture organic acid or mixture organic acid or mond or mond or formula I and another organic acrowth of mond or compound of Formula necessary to inhibit the around of organic acids or mixture. compound or Formula I and another organic acid or mixture in inhibit the growth of mold in ecompound or acids necessary to inhibit and common acids necessary to acids necessary to forganic acids necessary and anniving said common acids necessary and anniving said common acids feed common acid f 20 said feed composition; and applying said compound or acids or mixture of organic acid or mixture of organic acids organic acid or mixture of organic acids organic acids or mixture of organic acids or mixture or ordanic acids necessary to innibit the growth of me organic acids necessary to innibit the growth of organic acid composition; and applying mixture of organic said feed composition; acid or mixture of organic said feed and esid organic said or mixture of organic said feed tanders. Formula 1 and sald organic acid or mixture of organic acid concentration. In yet another embodiment of the present may of Formula in another embodiment of the present may in another embodiment of the present in any in a invention, the above-mentioned compounds of Formula 1 may

the above-mentioned compounds of Formula 1 may

the growth of mold in

the above-mentioned compounds of Formula 1 may

the growth of mold in

the above-mentioned compounds of Formula 1 may

the growth of mold in

the above-mentioned compounds of Formula 1 may

the growth of mold in

the above-mentioned compounds of Formula 1 may

the growth of mold in

the above-mentioned compounds of Formula 1 may

the above-mentioned compounds of Formula 1 may

the growth of mold in

the above-mentioned compounds of Formula 1 may

the above-mentioned compo 25 be used in a method for computing the concentration in said animal feed comprising 30 35

feed of a compound of Formula I necessary to inhibit the growth of mold in said animal feed; and applying said compound of Formula I to said feed in said concentration.

5

10

15

20

25

30

35

[0583] In yet another embodiment of the present invention, the above-mentioned compounds of Formula I and organic acids may be used in a method for inhibiting the growth of mold in animal feed comprising computing the concentration in said feed of a compound of Formula I and another organic acid or mixture of organic acids necessary to inhibit the growth of mold in said animal feed; and applying said compound of Formula I and said organic acid or mixture of organic acids to said feed in said concentration.

[0584] In yet another embodiment of the present invention, the above-mentioned compounds of Formula I may be used in a method for improving the mold resistance of an animal feed composition, the method comprising discontinuing the use of DL-methionine as a feed supplement; computing the concentration in said feed of a compound of Formula I necessary to inhibit the growth of mold in said animal feed; and applying said compound of Formula I to said feed in said concentration.

[0585] In yet another embodiment of the present invention, the above-mentioned compounds of Formula I and said organic acids may be used in a method for improving the mold resistance of an animal feed composition, the method comprising discontinuing the use of DL-methionine as a feed supplement; computing the concentration in said feed of a compound of Formula I and another organic acid or mixture of organic acids necessary to inhibit the growth of mold in said animal feed; and applying said compound of Formula I and said organic acid or mixture of organic acids to said feed in said concentration.

[0586] In yet another embodiment of the present invention, the above-mentioned compounds of Formula I may be used in a method for inhibiting mold in animal feed directly or indirectly making information available for

computing the concentration in said feed of said compound in said feed of said compound in said feed of said compound in said in said feed of said compound in said feed of said computing the concentration in said teed of said compound in said teed of said in said in said mold present in said mold present in format to inhibit mold present information in indirectly making information of Formula I necessary or indirectly or indirectly making information of Formula I and directly or indirectly making information of Formula I and directly or indirectly or indirectly making in a said to inhibit mold present in said to inhibit mold present in said of Formula I necessary to inhibit mold present in said in said making information in said and present in said of Formula I necessary to inhibit mold present in said

of Formula I necessary to indirectly making I to said

on indirectly or indirectly making I to said

on indirectly making of Formula I to sai animal reed; and directly or indirectly making information to said compound of Formula I to said applying said compound of Formula in said concentration available for concentration 10587] In yet another embodiment of the present and compounds of Formula I and mora in a method for inhihiting mora invention, and may be used in a method for inhihiting mora invention. Invention; the above-mentioned compounds of Formula I and the above-mentioned compounds of inhibiting mold in a method for inhibiting mold in a method for information are acids may be used in a method for information organic acids may be reactive or indirectly making information organic acids may be used in a mair and information organic acids may be used in a mair and information organic acids may be used in a method for inhibiting mold inhi organic acids may be used in a method for inhibiting mold information in a and feed directly or indirectly aking in a a a feed directly or concentration in a and feed in animal feed directly the concentration in a available for community or in a a method for inhibiting mold in a method for community in a a method for inhibiting mold in a method for community in a in animal teed directly or indirectly making information of asid feed of concentration in said feed of available for computing the concentration of available for computing the available for computing the concentration of mormila tank the concentration of animal available for computing of mormila tank the concentration of animal teed directly or indirectly making information of asid compound of mormila tank the concentration in animal teed directly or indirectly making information of asid concentration in said feed of animal teed directly or indirectly making information of asid concentration in said feed of animal teed directly or indirectly making information of asid concentration in said feed of animal teed directly or indirectly making information of asid concentration in animal teed directly or indirectly making information of asid concentration in animal teed directly or indirectly making information of asid concentration in animal teed directly or indirectly or indirectly making information of animal teed directly or indirectly making information or indirectly making infor feed in said concentration. available for computing the concentration in said feed of said and the concentration of said available for computing of organic acide necessary to said compound of mixture of organic acide necessary to sala compound or mixture of organic acids necessary to organic acid or mixture of organic acids necessary to organic acid or mixture of organic acid or more acid or mixture or more acid or more acid or mixture or more acid or mo inhibit mold present in said animal feed; and directly or applying said animal feed; and directly or mixture of information available for applying or mixture of indirectly making I and early organic acid or mixture indirectly making I and early organic acid or mixture of indirectly making I and early organic acid or mixture of indirectly making I and early organic acid or mixture of indirectly making I and early organic acid or mixture of indirectly making I and early organic acid or mixture of indirectly making I and organic acid or mixture of indirectly making I and organic acid or mixture or acid organic acid or mixture or acid o indirectly making information available for applying said or mixture of compound of Formula I and said concentration 5 compound of Formula 1 and said concentration.

said feed in said concentration. acaclas to sala reed in sala concentration.

The sala reed in sala concentration.

The sala reed in sala concentration.

The sala reed in sala concentration. [0588] In yet another embodiment of the present may of Formula I for for another embodiment of compounds of formula for for invention, the manufacture of a nutrient composition for the present may invention, the above-mentioned compounds of Formula I may interest composition for with the manufacture of a nutrient composition with the manufacture feed by treating early feed by treating early in animal feed by the feed by t be used in the manufacture of a nutrient composition for a nutrient composition feed with animal feed by treating said effective amount inhibiting mold in animal in an anti-mold effective animal in an anti-mold effective animal inhibiting mold in animal in an anti-mold effective amount inhibiting mold in animal in an anti-mold effective amount inhibiting mold in animal in an anti-mold effective amount inhibiting mold in animal in an anti-mold effective amount inhibiting mold in animal in an animal in animal in an animal in a inhibiting mold in animal feed by treating said reed with amount.

inhibiting mold in animal feed by treating effective amount.

inhibiting mold in animal feed by treating effective amount.

In an animal feed by treating said reed with amount.

In animal feed by treating said reed with amount.

In animal feed by treating said reed with amount. 20 sald nutrient composition in an anti-mold effective amount the nutrient composition may also the nutrient composition of the above-mentioned organic acids in another embodiment, the above-mentioned organic acids in another embodiment, the above-mentioned organic acids In another embodiment, the nutrient composition may also acids, comprise or more of the above-mentioned organic acids, comprise one or more of the above-mentioned organic acids, comprise one or more of the above-mentioned organic acids, comprise one or more of the above-mentioned organic acids, comprise or a mixture thereof mixture thereof. another embodiment of the present [U589] In yet another embodiment of the present may of Formula I feed of Formula feed compounds of an animal feed invention; the above-mentioned compounds in an animal feed invention; a method of inhihiting mold in a method of inhihiting mold inhihiting mold in a method of inhihiting mold inhihiting mol 15 Invention, the above-mentioned compounds of Formula I may mold in an animal feed in the method of inhibiting mold in an ethod of inhibiting monitoring the be used in a method common real method compounds of formula I may mold in an animal feed. De used in a method of innibiting monitoring the composition of method comprising ment in early fee concentration of methionine supplement in said feed

concentration of methional amounts of said methionine

composition, adding additional an anti-mold effective

composition, needed to achieve an anti-mold effective composition of methionine supplement in said feed concentration adding additional amounts of and additional amounts of an additional amounts of a second amount of a or a mixture thereof. 20 composition, adding additional amounts of said feed to achieve an anti-mold effective supplement as needed to achieve an arrangement in said feed supplement as needed to achieve an arrangement in said feed to achieve an arrangement as needed to achieve arrangement as needed to achieve an arrangement as needed to achieve arrangement as needed to achieve an arrangement as needed to achieve an arrangement ar supplement as needed to achieve an anti-mold effective in said feed supplement in said feed concentration of methionine supplement in said feed In yet another embodiment of the present invention:

in a method of anhancing the present may

invention:

in a method of anhancing the majatahility of animal invention. invention, the above mentioned compounds or rormula 1 may the above mentioned compounds or rormula 1 may of animal the palatability of animal the palatabili 25 De used in a method comprising treating the food with a food, the method comprising treating the food, composition. 30 35

compound of Formula I in an amount sufficient to give a food of formula I in an amount sufficient to give a food of formula I in an amount sufficient to give a food of food of the compound of formula I in the food of food of food of the compound of the compound of food of the compound of food of the compound of the compound of food of the compound of the c compound of Formula I in an amount sufficient to give a of preferant to give a of the compound of Formula I in the food of the compound of Formula I in the preferant to give a preferant to give a preferant to give a compound of the compound of Formula I in the food of the compound of Formula I in an amount sufficient to give a preferant to give a compound of Formula I in an amount sufficient to give a compound of Formula I in the food of the compound of Formula I in an amount sufficient to give a compound of Formula I in the food of the compound of Formula I in an amount sufficient to give a compound of Formula I in the food of the compound of Formula I in an amount sufficient to give a compound of Formula I in the food of the compound of Formula I in the comp concentration of the compound of Formula I in the tood of the compound of the com between about 0.01 wt. \* and about 0.5 wt. \*. preserably of aquaculture. I in the common of formula I in the common of the commo tood is food for canines, telines, or aquaculture. For the compound of Formula 1 in 16%.

tood is food for canines, telines, or aquaculture. For the formula 1 in 16%. dogs, the concentration of the compound of Formula I in the compound of Formula 0.15%;

the concentration of the compound of Formula 0.15%;

the concentration of the compound of Formula 1 in Tood is preterably between about 0.05% and about 0.30%.

Tood is preterably between about normalis Time normalis and normalis to the normalis and normalis for math done and normalis to the n TOr cats, lt ls prererably perween about v.zu\* and is

TOr both dogs and cats, the compound of Formula I is

For both times or nim the compositions or the compositions or a sair in a preferred embodiment, HMRA or a sair instant in a preferred herein romarise HMRA or a sair instant in a preferred herein romarise HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a preferred embodiment, HMRA or a sair instant in a sair [0591] In a preferred embodiment, HMBA, acid selections comprise HMBA, acid selections comprise the compositions described herein first organic acid selections combinations described herein and a first organic acid selections. ester or amide thereof; and a formic acid, actic acid, from the group consisting of tormic acid, lactic acid.

from the group butyric acid, mandelic acid, citric acid.

propionic acid, tartaric acid, mandelic acid. preferably IMBA or DLM. proplonic acid, butyric acid, mandelic acid, citric acid, mandelic acid, aurcinic acid, horic acid, aurcinic acid, horic acid, arid, arid, arid, acid, horic acid, arid, arid, acid, horic acid, acid, horic acid, 5 malic acid, tartaric acid, mandelic acid, succinic acid, boric acid, succinic aren mandelic acid, succinic aren mandelic acid, succinic aren mandelic acid, boric acid, and aluraric acid, and aluraric acid, and aluraric acid, alveolic acid, and aluraric acid, aluraric acid, adipic acid. rumaric acid, glycolic acid, and glutaric acid, the first organic acid, the first organic acid, and glycolic acid, and acid, is aplented from the acid, the first organic acid, the first organic acid, acid, the first organic acid, acid, the first organic acid, adipic acid, the first organic acid armin and preferably, preferably, armin armin and acid armin preferably, the first organic acid, propionic acid, butyric group consisting of formic acid, propionic acid, acid 20 105921 In various preferred embodiments, an acidulant comprise an acidulant of mineral acide

compositions or combinations faring of mineral acide

compositions or rown consisting of mineral acide gelected from the group consisting of mineral acids of phosphoric acid hydrochic compositions or combinations further comprise an acids, compositions of combinations further of mineral acids, compositions of combinations from the group consisting of mineral acids, compositions of combinations from the group consisting of mineral acids, compositions of combinations for the group consisting of mineral acids, compositions of combinations for the group consisting of mineral acids, compositions of combinations for the group consisting of mineral acids, compositions of combinations for the group consisting of mineral acids, compositions of combinations for the group consisting of mineral acids, compositions of combinations for the group consisting of mineral acids, compositions of combinations for the group consisting of mineral acids, compositions of combinations for the group consisting of mineral acids, compositions of combinations of combinations of combinations for the group consisting of mineral acids, compositions of combinations of combi preferably selected from the group consisting of phosphoric acid, hydrochloric acid, phosphorous acid, second organic acid, and nitric acid, hydrobromic acid, and nitric acid, hydrobromic acid. acid, and lactic acid. acid, sulturic acid, and nitric acid; a second organic acid, hydrobromic acid, aroun consisting of formic acid, and nitric acid, and nitric acid, aroun consisting of formic acid, around 15 hydrobromic acid, and nitric acid; a second organic acid, acetic acid; a formic acid, acid, actic acid, and nitric acid; a formic acid, acid, actic acid, ac selected from the group consisting of formic acid, lactic acid, benzoic acid, citric acid, benzoic acid, citric acid, propionic acid, rarraric acid, mandalic acid, acid, malic acid, mali acid, malic acid, sorbic acid, boric acid, sorbic acid, acid, sorbic acid, aci acid, malic acid, sorbic acid, and alutaric acid, tumaric acid, ac acid, sorbic acid, boric acid. In even more acid, sorbic acid, and glutaric acid. and serond acid, and serond acid, acid, acid, first organic acid, and serond acid, acid, sorbic acid, first organic acid, and serond acid, acid, sorbic acid, sorbic acid, and serond acid, acid, acid, sorbic acid, sorbic acid, sorbic acid, and serond acid, acid, and serond acid, sorbic acid, and serond acid, sorbic acid, sorb 20 adipic acid, glycolic acid, the first organic from the armin the preferred embodiments, and are indemendently selected from the preferred embodiments. organic acid are independently selected from the group butyric acid, butyric acid, propionic acid, selected from the group acid, butyric acid, propionic acid, selected from consisting of formic acid, third organic acid, and/or a rhird organic acid. consisting of formic acid, proplonic acid, butyric acid, and lactic acid; and/or a third organic acid, propionic acid, acid; and/or a third organic acid, malic acid.

the group consisting of formic acid, lactic acid, malic acid, the group acid henzoic acid. 25 the group consisting or formic acid, lactic acid, malic acid, benzoic acid, acid, benzoic acid, acid, acid, acid, benzoic acid, benzoic acid, butyric acid, acid, acid, acid, acid, acid, acid, butyric acid, acid 30 35

```
enrhin anid horin anid acid, citric acid, fumaric acid, tanin anid acid,
                                                                                                                                                                                                                                                                                                                                                                                                                                                  sorbic acia, manaelic acia, citric acia, iumaric a acid, adipic acid,
                                                                                                                                                                                                                                                                                                                                                                                                                                        glycolic acid, and glutaric acid.
                                                                                                                                                                                                                                                                                                                                        5
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Compositions and still more preferred embodiment, the formal and nhoanhoric acid
                                                                                                                                                                                                                                                                                                                                                                                                       Compositions and combinations described herein comprise of hmma is from about 5 to about the content of hmma is from about 5 to about the content of hmma is from about 5 to about the about the content of hmma is from about 5 to about the about th
                                                                                                                                                                                                                                                                                                                                                                                           Preferably, the content of HMBA is from about 5% to about 5% to about 5% of acid.
                                                                                                                                                                                                                                                                                                                                                                                 Preferably, the content of the sum of the HMBA, formic acid, propionic acid, a
                                                                                                                                                                                                                                                                                                                                                                       10
                                                                                                                                                                                                                                                                                                                                                             is from about 65% to about 85% of said sum; the content of the formic acid of the formic 
                                                                                                                                                                                                                                                                                                                                                   the propionic acid is from about 85% of said sum; the content of the inner of the order of said sum; the content of the inner of the content 
                                                                                                                                                                                                                                                                                                                                       the propionic acid is from about 1s to about 15s of said is from about 1s to a
                                                                                                                                                                                                                                                                                                                            Sum; and the content of the phosphoric acid is from about 10% of said sum. Even more preferably, the content of the phosphoric acid is from about 10% of said sum. the content of the phosphoric acid is from about 10% of said sum. the content of
                                                                                                                                                                                                                                                                                                                  Content of MMBA is about 10° of said sum. Even more preferably, the said sum; the content of content of
                                                                                                                                                                                                                    15
                                                                                                                                                                                                                                                                                                      the formic acid is about 10% of said sum; the content of said sum; the said sum; the content of said sum; the content of said sum; the said sum
                                                                                                                                                                                                                                                                                            the propionic acid is about 75% of said sum; the content of said sum; and the content
                                                                                                                                                                                                                                                                                  of the phosphoric acid is about 20 of said sum; and cure bropionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is about 50 of said sum; and cure propionic acid is acid is acid is acid sum; and cure propionic acid is a
                                                                                                                                                                                                                                                                      Alternatively, the content of HMBA is from about 200 to to rha form; about is
                                                                                                                                                                                                                                                             about 40% of said sum, the content of HMBA is from about 20% to the formic acid is
                                                                                                                                                                20
                                                                                                                                                                                                                                                   and the content of the nhoanhoric about 55 to about 10 to about of the nhoanhoric about 200 content of the nhoanhoric arial is grown.
                                                                                                                                                                                                                             propionic
and the content of the phosphoric acid is from about 10 to about 200 of sala sum,

and the content of the phosphoric acid is from about 10 to acid is from aci
                                                                                                                                                                                                                   about 15% of said the phosphoric acid is trom about 1% to from the content of
                                                                                                                                                                                                       ADOUT 15% OF SAIR SUM; even more preferably, the content of the formic content c
                                                                                                         25
                                                                                                                                                                                             ACIQ is about 30% of said sum, the content of the formic of the propionic
                                                                                                                                                                                  acid is about 55% or said sum, the content of the property of the
                                                                                                                                                                      phosphoric acid is about 50 of said sum.
                                                                                                                                                                                                                    ID594]

In another more preferred embodiment, the
                                                                                                                                                  Compositions In another more preferred embodiment, the and nhosnhoric acid.
                                                    30
                                                                                                                                        HMBA, butyric acid, lactic acid, and phosphoric acid.

one of the companions of the 
                                                                                                                              treterably, the content or MMBA is from about 20% to about the content: the content: the content of the Albaria acid, lactic acid, and is the content of the content.
                                                                                                      phosphoric acid content; the content of the butyric acid, and from ahour acid is
                                                                                             phosphoric acia content; the said sum; the butyric acia is from about 10% to about 30% of said sum; the butyric acia is from ahour, in a said sum; the content of the butyric acia is from ahour, in a said sum; the content of the butyric acia is and the said sum; and
35
                                                                                 the content of the from about 10° to about 30° of said sum; the content of the from about 10° to about 30° of said sum; and
                                                                         Lactic

the content of the phosphoric acid is from about 30% of sala sum;

or asid and more preferably the 20% to
                                                             the content of the phosphoric acia is from about 20% to said sum. Even more preferably, the content of
```

HMBA is about 30% of said sum; the content of the butyric acid is about 20% of said sum; the content of the lactic acid is about 20% of said sum; and the content of the phosphoric acid is about 30% of said sum. Alternatively, the content of HMBA is from about 20% to about 40% of said sum of the 2-hydroxy-4-(methylthio) butanoic acid, butyric acid, lactic acid, and phosphoric acid content, the content of the butyric acid is from about 5% to about 25% of said sum, the content of the lactic acid is from about 10% to about 30% of said sum, and the content of the phosphoric acid is from about 25% to about 45% of said sum; more preferably, the content of HMBA is about 30% of said sum, the content of the butyric acid is about 15% of said sum, the content of the lactic acid is about 20% of said sum, and the content of the phosphoric acid is about 35% of said sum.

5

10

15

20

25

30

35

In yet another more preferred embodiment, the compositions and combinations described herein comprise HMBA, butyric acid, formic acid, lactic acid, and phosphoric acid. Preferably, the content of HMBA is from about 10% to about 30% of the sum of the HMBA, butyric acid, formic acid, lactic acid, and phosphoric acid content; the content of the butyric acid is from about 2% to about 22% of said sum; the content of the formic acid is from about 20% to about 40% of said sum; the content of the lactic acid is from about 8% to about 28% of said sum; and the content of the phosphoric acid is from about 10% to about 30% of said sum. Even more preferably, the content of HMBA is about 20% of said sum; the content of the butyric acid is about 12% of said sum; the content of the formic acid is about 30% of said sum; the content of the lactic acid is about 18% of said sum; and the content of the phosphoric acid is about 20% of said sum.

[0596] In yet another more preferred embodiment, the compositions and combinations described herein comprise HMBA, butyric acid, lactic acid, propionic acid, and phosphoric acid. Preferably, the content of HMBA is from

about 10% to about 30% of the sum of the HMBA, butyric acid, lactic acid, propionic acid, and phosphoric acid content; the content of the butyric acid is from about 2% to about 22% of said sum; the content of the lactic acid is from about 8% to about 28% of said sum; the content of the propionic acid is from about 20% to about 40% of said sum; and the content of the phosphoric acid is from about 10% to about 30% of said sum. Even more preferably, the content of HMBA is about 20% of said sum; the content of the butyric acid is about 12% of said sum; the content of the lactic acid is about 18% of said sum; the content of the propionic acid is about 30% of said sum; and the content of the phosphoric acid is about 20% of said sum.

5

10

15

20

25

30

35

In yet another more preferred embodiment, the [0597] compositions and combinations described herein comprise HMBA, butyric acid, formic acid, propionic acid, and phosphoric acid. Preferably, the content of HMBA is from about 1% to about 20% of the sum of the HMBA, butyric acid, formic acid, propionic acid, and phosphoric acid content; the content of the butyric acid is from about 1% to about 15% of said sum; the content of the formic acid is from about 65% to about 85% of said sum; the content of the propionic acid is from about 1% to about 15% of said sum; and the content of the phosphoric acid is from about 1% to about 15% of said sum. Even more preferably, the content of HMBA is about 10% of said sum; the content of the butyric acid is about 5% of said sum; the content of the formic acid is about 75% of said sum; the content of the propionic acid is about 5% of said sum; and the content of the phosphoric acid is about 5% of said sum.

[0598] In yet another more preferred embodiment, the compositions and combinations described herein comprise HMBA, formic acid, and propionic acid. Preferably, the content of HMBA is from about 20% to about 40% of the sum of the HMBA, formic acid, and propionic acid content; the content of the formic acid is from about 40% to about 60% of said sum; and the content of the propionic acid is from

about 10% to about 30% of said sum. Even more preferably, the content of HMBA is about 30% of said sum; the content of the formic acid is about 50% of said sum; and the content of the propionic acid is about 20% of said sum.

5

10

15

20

25

30

35

[0599] In yet another more preferred embodiment, compositions and combinations described herein comprise HMBA and phosphoric acid. Preferably, the content of the HMBA is from about 5% to about 50% of the sum of the HMBA and phosphoric acid content. In various more preferred embodiments, the content of the HMBA is about 5%, or about 10%, or about 15%, or about 20%, or about 25%, or about 30%, or about 35%, or about 40%, or about 45%, or about 50% of said sum.

[0600] In another embodiment of the present invention is provided an animal feed composition comprising a compound of Formula I as described herein, and an acidulant as described herein.

[0601] In another embodiment of the present invention is provided a method of inhibiting or killing microbes in a subject, comprising treating said subject with a composition or combination as described herein. In a preferred embodiment, the subject to be treated is water or food, preferably selected from the group consisting of human food, livestock food, pet food, or aquaculture food.

[0602] Animals for which the food, food ingredients and/or feed compositions described herein may be provided include humans, ruminants such as dairy cows, lactating dairy cows, dairy calves, beef cattle, sheep, and goats; aquaculture such as fish and crustaceans (including, but not limited to, salmon, shrimp, carp, tilapia and shell fish; livestock such as swine and horses; poultry such as chickens, turkeys, and hatchlings thereof; and companion animals such as dogs and cats.

[0603] The exact formulation of the above-mentioned animal feed composition is not critical to the present invention. Feed ingredients are selected according to the nutrient requirements of the particular animal for which

the feed is intended; these requirements depend, inter animal these requirements depend, the animal commons of the reed 1s intended; these requirements depend, inter reed 1s intended; the age and stage of development reed intended; intended and stage of fantore read intended; the age and other fantore read intended; the animal read intended; the age and other fantore read intended; the animal read intended; the age and other fantore read intended; the age and other read intended; the age and animal read intended; the alia, upon the age and stage of development of the animal, see of the animal, and other factors. The hadis of the animal, the sex of the armined into eight of the armined int the sex of the animal, and other factors. Feed ingredient the basis of their formulation diefa. Any formulation diefa. may be grouped into eight classes on the basis of their forages in formulating diets: fan frach.

composition and their use range night and forage for any roughages. composition and their use in formulating diets: dry fresh; range plants and forages fed fresh; range plants and mineral aunniementa; mineral and roughages; pasture; protein aunniementa; mineral and roughages; energy feeds: and rougnages; pasture; range plants and rorages red range plants and rorages red and rougnages; protein supplements; and addition and addition as allages; silages; aritemin annotations and additional annotations and additional annotations. slages; energy reeds; protein supplements; and additives. profession supplements; and additives. profession supplements; curronmitto on professional pages role of the supplements role of the s supplements; vitamin supplements; and additives. See

(U.S.) Subcommittee on Feed

(U.S.) Subcommittee of Pand

National Research crafted craf Naclonal research Council (U.S.) Subcommittee on reed of Feed of Tour Composition, and row National Academy process of the Composition and row of the Composition of the Composition and row of the Composition of the Composi Composition, United States-Canadian Tables of Feed 2, 145

Composition, Whited States-Canadian Academy Press, pp. 2, 145

arbitrary arbitrary are to a certain extent.

Composition, These classes are to a certain extent. Composition 3d rev., National Academy press, pp. 2, 145

National (1982). These classes are, to a certain extent, arout than denomination will also denominate than the second secon as some feed ingredients could be classified in more depend will also depend a feed formulation will also depend a feed formulation ingredient with each ingredient one class. Typically, ated with each ingredient one class. Costs associated with each ingredient one class. Typically, a feed formulation will also depend ingredient, which dives the upon the costs associated with a ingredient which dives the upon the costs associated with ingredient which dives the upon the costs associated with ingredient which dives the upon the costs associated with a cost as a upon the costs associated with each ingredients which gives the least-expensive heims the neeferred formulation least-expensive heims the neeferred formulation least-expensive heims the neeferred formulation least-expensive heims the neefer hei 5 least-expensive composition of ingredients which greferred formulation.

needed nutrients being formulation formulation. [0604] Silage is a forage product that is produced forage and fermentation of green forage and fermentation of mhead arona the harvest, and arain anorthism nlanta from the harvest, and arain anorthism along another anorthism and arain anorthism and arona anorthism anorthism. trom the narvest, storage and termentation of green torage and termentation of the arain is ready for the narvest, and grain hefore the arain is ready for crops such as corn and all hefore the arain is ready for are chonned are chonned crops such as corn and grain before the grain is ready for are chopped, stems and all, harvest. The niant material is atored in all harvest. 10 are chopped, stems and all, before the grain is ready to storage in silos, storage i narvest. The plant material is stored in silos, storage the material to navest bunkers or covered piles causing the material the national the nation bags, bunkers or covered piles causing the material to plant the plant the ph and preserving is anhier the ph and preserving the ph and preserving the ph and forace is anhier thereby lowering the ph and preserving the ph and forace is anhier thereby thereby the ph and preserving the plant the ph and preserving the rerment, thereby lowering the ph and preserving the plant the ph and preserving the ph and preserving the ph and preserving the ph and preserving the plant thereby lowering the ph and preserving the plant the ph and preserving the ph and preserving the plant the ph and preserving the plant the ph and preserving the plant the ph and preserving the ph 15 langes in phi temperature feed formulations depend in [10605] material until it can be red. The englied forage if the temperature food formulations do to changes in phi to changes in phi to change in the change Lubusi As noted above, of development of the animal to of development of the animal to of development of the chicken are part upon the age and summers (Nutrition of the chicken and the fed. The sand Summers of the fed. The fed. The fed. The fed. The fed. part upon the age and stage of development of the chicken, several part upon the age and summers (Nutrition of the chicken, several part upon and summers (Nutrition of the chicken, several part upon the age and stage (Nutrition of the chicken, several part upon the age and stage of development of the chicken, several part upon the age and stage of development of the animal to the chicken, several part upon the age and stage of development of the chicken, several part upon the age and stage of development of the chicken, several part upon the age and stage of development of the chicken, several part upon the age and stage of development of the chicken, several part upon the age and stage of development of the chicken, several part upon the age and stage of development of the chicken, several part upon the age and stage of development of the chicken, several part upon the age and summers of the chicken, several part upon the age and summers of the chicken of the chicke be fed. Leeson and Summers (Nutrition of the Chicken, broilers broilers)

be fed. Leeson and University Books (2001))

ed., pp. 502-510, noultry diets for nullets.

renresentative noultry ed. Pp. 502-510, University Books (2001) layers, diets for pullets, mar chicken diets representative poultry for example, most chicken diets and broiler hreeders 20 representative poultry diets for pullets, most chicken wheat and broiler preceders. and proller preeders. For example, most contain oats, wheat, and proller preeders. The example of a contain energy concentrates such as an or an contain energy concentrates such as such as soybean meal, afflower barley, or sorghum; protein neaming apparent aparticles apparent appare parley, or sorghum; brotein sources ender as solveen mean or sorghum; brotein bources ender animal brotein animal brotein animal order of the other etc.) other ollseed meals (e.g., peanut, animal protein sources of the sources animal protein sources animal protein arain animal protein arain sources and meal, animal protein arain arain meal, arain mea 25 sunflower, etc.), cottonseed meal, animal protein grain fish meal, etc.), grain (meat and bone meal, dried whey) 30 35

legumes (e.g., and mineral supplements) if necessary (for and viramin and mineral supplements) legumes (e.g., and mineral supplements, in calcium and mineral supplements, in calcium and mineral supplements, in calcium and vitamin and hone meal is high in calcium and inetance meat and witamin meat and hone meal is high in calcium and inetance meat and hone meal is high in calcium and hone meat and hone meal is high in calcium and hone meat and and Vitamin and mineral supplements, in calcium and instance, meat and bone meal is high in calcium and instance, meat and there minerals do not need to these minerals do not need to these minerals do not need to the instance, and thus these minerals do not need to the instance, and thus these minerals do not need to the instance, and thus these minerals do not need to the instance, and thus these minerals do not need to the instance, and thus the instance is not need to the instance. instance, meat and bone meal is high in calcium and be minerals do not need to be minerals do not need home meat and thus these minerals in ind meat and thus phosphorous, in a feed ration containing meat and thus phosphorous, in a feed ration prosphorous and thus these minerals do not need to be meat and bone and thus these minerals do not need to be meat and bone these minerals do not need to be supplemented in a feed ration containing meat ingredients in meat ingredients of the different ingredients of the modulation at any of the modulation at any of the meating meat in narr unon the meating meat in narr unon the meating feed denomine in narr unon the modulation of the modulation at any of the modulation o meal). The relative amounts of the different ingredients in part upon the production while poultry feed depends in part inner in nrotein. While the hird grarter rations are higher in nrotein. poultry feed depends in part upon the production while around the bird. Starter rations are higher in protein around the bird. Finisher feeds can be lower in arotein around the bird. the bird. Starter rations are nigher in protein awine grower and finisher remire leas northein model diera for awine grower and finisher less protein. Model diets for swine and model diets for swine and model diets for swine and model diets modified and may be modified older birds require also available other animals are also available. older birds require less protein. Model diets for swine and may be modified available, and may be modified to other animals are also available, of the animals the next out and other animals are also available. other animals are also available, and may be modified be animal(s) to be according to the particular needs of the animal(s) The term "inhibit" when used herein in phrases [0606] The term "inhibit" when used herein in phrases any one or more of the pacteria" means any one in arouth of the such as "inhibiting bacteria" have decrease in arouth of the such as harteria or mold. (h) any decrease in arouth of the such as harteria or mold. such as "inhibiting bacteria" means any one or more of the any decrease in growth of colony have bacteria or mold; (b) any decrease in terms of colony have measured in terms of colony have bacteria or mold; which may be measured in terms of colony have been as a sure of the measured in terms of colony have been as a sure of the measured in terms of colony have been as a sure of the measured in terms of colony have been as a sure of the measured in terms of colony have been as a sure of the means any one or more of the colony have been as a sure of the means any one or more of the colony have been as a sure of t 5 Killing bacteria or mold; (b) any decrease in growth of the measured in terms of harteria bacteria or mold; be measured in terms harteria bacteria or mold; be measured in the concentration of harteria bacteria. (c) any decrease in the concentration of harteria bacteria. pacteria or mold, which may be measured in terms of bacteria or mold, which may be measured in terms of bacteria or mold, which may be measured in terms of bacteria or mold, the inahility of harraria or mold, the inahility of harraria or mold, the inahility of harraria or mold. Or mold. Or mold. Or counts; (c) any decrease in the concentration of bacteria or mold to grow the inability of bacteria or here may be counts; or mold; or mold; as a serious medium rach of these may be counts; or mold; as a serious medium as a serious medium as a serious medium. or mold; or (d) the inability of bacteria or mold to gro

or mold; or (d) the inability of bacteria or the harterial or

on a particular selection har commaring the harterial or

determined for 10 on a Particular selection medium. Each of the bacterial or more of the determined, for instance, or concentration of the methods of fungal colony areance of the annight of the areance of the arrageon in the areance of the arrageon of the arrageo fed. fungal colony counts or concentration of pacteria or mold of the methods of of the application or fundal colony fundation of the harterial or fundation or fundat present in the absence of the bacterial or fungal colony the present invention of hanteria or mond after the present invention of hanteria or mond after 15 the present invention with the bacteria or mold after counts or concentration of bacteria or mold after counts or concentration of pacteria or mold after invention.

counts or concentration of methods of the present functional application of anirable hasterial dea or functional application of anirable hasterial dealers. application of the methods of the present invention. Will application of suitable bactericides or colonic colonic application of suitable present in colonic c a ten-fold difference in colony counts. invention call

a ten-fold difference in colony free present in of a

[0607]

[0607]

[0607] Application of sultable pactericides or rungi, counts.

Application of sultable pactericides in colony counts. [U6U1] Certain methods of the present invention, of a the concentration, for instance, animal feed necessary to computing, for instance, animal feed necessary to computing, for in an animal feed necessary. 20 tor computing tor instance, the concentration, or a tor computing tor instance, the concentration, or a animal feed, necessary to compound of Formula I in an animal in an animal feed compound of Formula I in an animal in an animal feed compound of Formula I in an animal in an animal feed compound of Formula I in an animal in an animal feed compound of Formula I in an animal in an animal feed compound of Formula I in an animal in an animal feed compound of Formula I in an animal feed compound of Formula I in an animal in animal in an animal in animal compound of Formula I in an animal teed, necessary to the inhibit bacteria or mold present in an animal another inhibit bacteria or mold present in an animal another concentration of a compound or formula I and another or mold to inhibit bacteria or regard or acids necessary to inhibit herein are reasonable organic acid or animal feed provided herein are reasonable organic in an animal feed provided herein are reasonable organic in an animal feed provided herein are reasonable or acids feed provided herein acids or acids feed provided herein are reasonable or acids and acids are reasonable or acids are rea Innibit pacteria or mold present in an animal reed, of concentration of a compound of remarks to inhihit hactoria concentration of a concentration organic acid or acids necessary to inhibit bacteria or mold herein are Examples 1.

Present in an animal securite of compound of rormal are present in an inhibit bacteria or mold herein are Examples 1. 25 present in an animal reed. Provided nerein are Examples

of compound of Formula I

and of the order order of animal second order ord which illustrate amounts of compound of Formula I inhibit are sufficient to inhibit and or organic acids that are sufficient to inhibit and or other organic acids that 30 35

bacteria or mold. Also provided hereinabove and hereinbelow are acceptable ranges of amounts of compound of Formula I and/or other organic acids, and ratios between the two, which are suitable for use the methods of the present invention. Other suitable concentrations, ranges and ratios can be determined as needed.

Treatment of the animal feed compositions with the compounds of Formula I and optionally with the other organic acids disclosed herein, or with the compounds of Formula I and optionally with other organic acid(s), may be done by mixing the compound of Formula I (and other organic acid, if present) with the other ingredients in the feed, such as the corn, soybean meal, other feed supplements, etc., as the feed is being formulated. Alternatively, the compound of Formula I and optional other organic acid(s) may be applied to a pre-mixed or pre-pelleted feed. In either case, the compound of Formula I and optional organic acid(s) are preferably added as liquids, and uniformly disperse throughout the bulk of the feed composition when applied. When the compound of Formula I and another organic acid are both used in the methods of the present invention, preferably said compound of Formula I and said other organic acid or acids are mixed together before application to the animal feeds. This pre-mixed compound of Formula I/organic acid(s) blend can be applied to the animal feed ingredients during formulation of the feed compositions, or can be applied to pre-mixed or pre-pelleted feed.

[0609] The term "cfu" stands for colony forming units.

[0610] The following examples illustrate the invention.

## EXAMPLE 1

5

10

15

20

25

30

35

[0611] The effects of increasing quantities of formic acid and/or Alimet® on the colony counts of four bacteria (E. coli, S. enteritidis, L. plantarum and C. jejuni) were studied. Varying amounts of formic acid or Alimet® were

added individually to cultures of these bacteria at pH 4.5 or 6.75 and the cultures were incubated for a length of time, whereupon colony counts were performed.

5

10

15

20

25

30

35

[0612] The S. enteritidis culture for the in vitro study contained a mixture of S. enteritidis ID-Lelystad (nalidixic acid resistant strain) and S. enteritidis (97.07773 RIVM, isolated from poultry). The E. coli culture contained a mixture of E. coli 0149K91K88 (VA2000-08915, pig pathogen) and E. coli ATCC 25922. The L. plantarum culture studied was L. plantarum Bd 99.00553. The C. jejuni culture studied was C. jejuni C356, ex. ID-Lelystad.

[0613] S. enteritidis and E. coli from fresh overnight cultures in Brain Heart Infusion broth were incubated aerobically in phosphate buffered (0.11 M) salt solution (8.5 g/L NaCl) with peptone (1 g/L), except for S. enteritidis at pH 4.5. For this culture, medium 5 was used as the broth, and the culture was incubated aerobically for 4 hours at 37°C. Colony counts were performed according to standard operating procedures.

[0614] The fresh overnight culture of *L. plantarum* in brain heart infusion broth were used to inoculate medium 5. The test tubes were incubated under reduced oxygen atmosphere for 6 hours at 37°C. Colony counts were performed.

[0615] C. jejuni grown on Campylobacter blood-free selective agar was used for inoculation. Preston broth was incubated under reduced oxygen atmosphere for 6 hours at 37 °C. Colony counts were performed.

[0616] Formic acid and Alimet® were added to the bacterial cultures in concentrations of 0.108 g/L, 0.30 g/L and 0.83 g/L. These dosages were chosen based on commercial use of Alimet® and an approximate 10-fold dilution in the proximal digestive tract.

[0617] A summary of the results obtained with formic acid and Alimet<sup>®</sup> on S. enteritidis and E. coli is given in Tables 1 and 2, and on L. plantarum and C. jejuni in Tables 3 and 4; the results are illustrated in Figures 1-4.

# Table 1

Effect of formic acid and Alimet® on population of S. enteritidis after 4 hours at pH 4.5 and 6.75

initial colony count: 5.23 log cfu/mL

5

10

15

20

25

Acid (g/L)		pH = 4.5		pH = 6.75		
Alimet®	Formic	log cfu/mL	Δ log	log cfu/mL	Δ log	
_	_	5.03	-0.20	6.62	1.42	
0.108	_	4.92	-0.31	6.71	1.51	
0.30	-	4.96	-0.27	6.63	1.43	
0.83		4.93	-0.30	6.53	1.33	
_	0.108	5.04	-0.19	6.79	1.59	
_	0.30	4.96	-0.27	6.77	1.57	
_	0.83	4.86	-0.38	6.72	1.52	

# Table 2

Effect of formic acid and Alimet $^{\$}$  on population of *E. coli* after 4 hours at pH 4.5 and 6.75

initial colony count: 5.24 log cfu/mL

Acid (g/L)		pH = 4.5		pH = 6.75	
Alimet®	Formic	log cfu/mL	Δ log	log cfu/mL	Δ log
_	_	5.36	0.12	7.47	2.23
0.108	_	5.45	0.21	7.33	2.09
0.30	_	5.25	0.01	7.36	2.12
0.83	_	3.96	-1.28	7.39	2.15
_	0.108	5.19	-0.05	7.48	2.24
_	0.30	4.96	-0.28	7.50	2.26
	0.83	5.08	-0.16	7.49	2.25

## Table 3

Effect of formic acid and Alimet® on population of L. plantarum after 6 hours at pH 4.5 and 6.75

initial colony count: 5.04 log cfu/mL  $\Delta$  log = log<sub>sample</sub> - log<sub>initial</sub>

5

10

15

20

25

30

Acid (g/L)		pH = 4.5		pH = 6.75	
Alimet®	Formic	log cfu/mL	Δ log	log cfu/mL	Δ log
_	_	5.67	0.63	6.10	1.06
0.108	_	5.67	0.63	6.09	1.05
0.30	_	5 <b>.57</b>	0.53	6.20	1.16
0.83	_	5.74	0.70	5.70	0.66
_	0.108	5.75	0.71	5.88	0.84
_	0.30	5.74	0.70	6.23	1.19
	0.83	5.56	0.52	6.19	1.15

### Table 4

Effect of formic acid and Alimet® on population of *C. jejuni* after 6 hours at pH 4.5 and 6.75

initial colony count: 5.23 log cfu/mL

Acid (g/L)		pH = 4.5		pH = 6.75		
Alimet®	Formic	log cfu/mL	Δ log	log cfu/mL	Δ log	
_		3.70	-1.53	6.54	1.31	
0.108	0.108 - 0.10		4.07	-1.16	6.44	
0.30	_	0.30	3.95	-1.28	6.40	
0.83	_	0.83	2.80	-2.43	6.34	
-	0.108	3.86	-1.37	6.27	1.04	
_	0.30	2.63	-2.60	6.38	1.15	
_	0.83	< 1.30	< -3.93	6.25	1.02	

[0618] S. enteritidis: Prior to inoculation, the S. enteritidis cultures had a colony count of 5.03 log cfu/mL at pH 4.5, and of 6.62 at pH 6.75. The results obtained at both pH values were similar for Alimet® and formic acid: at

PH 4.5, neither had a significant effect on inhihition of s pH 4.5, neither had a significant effect on inhibition of s.

growth of s. enteritidis;
enteritidis was observed. Itldis was observed. to inoculation, at pH 4.5, the following country for a rollow country fo 106191 E. coll: prior to inoculation, at PH 4.5, the at it was 7 47 to 2000 to 1000 to 5.36 log cfulminately a colony count of 5.36 approximately a colony count of ave approximately a coli cultures had a n 83 ml. Nimet® may approximately a coli cultures and n 83 ml. Nimet® may approximately a coli cultures 7 47 E. coli cultures had a colony count of 5.36 log cfu|mLi at a colony co pH 6.75, it was 7.47. Coli growth at pH 4.5, compared to coli growth at pH 4.5, compared to 1.3 log reduction of 1.3 reductio enteritidis was observed. the approximately 0.1 log reduction by the same concentration of formic acid at pH 4.5. Lower showed concentrations of both Alimet® and formic acid showed acid at pH 4.5. Lower acid showed acid showed acid showed acid showed acid at pH 4.5. Lower acid showed acid sh the approximately v.l 109 reduction by the same concentration of formic acid at ph 4.5. Lower concentration of formic acid at ph 4.5. concentrations of both Alimet and formic acid showed

concentrations of both Neither Alimet nor formic acid

inhibited R. coli at nH 6 75 olted E. Coll at Ph 6.75.

To inoculation, at ph 4.5, to inoculation, at ph 4.5, to inoculation, at ph 1.7, to inoculation, at ph 1.7, to inoculation, at ph 1.7, to inoculation, at ph 4.5, to inoculation, at ph 4.5, to inoculation, at ph 4.5, to inoculation, at ph 1.7, to inoculation, at ph 1.5, to inoculation, at ph 106201 L. plantarum. prior to inoculation at pH 4.5

106201 L. plantarum cultures had a colony count of 5.67 nor form
the L. plantarum cultures had a Neither Alimete nor form
cfulmic at nH 6.75. it was 6.10 the L. plantarum cultures had a colony count of 5.67 log nor formic the L. plantarum cultures 6.10. Neither nu armain at either nu armain at cfu/mL; acid inhibited L. nlantarum at either nu armain at acid inhibited L. 5 inhibited E. coli at pH 6.75. innipited L. plantarum at elther ph studied.

to inoculation, at ph 4.5, inoculation, at ph 1.7, inoculation, at ph 1.7, inoculation, at ph 1.7, inoculation, at ph 1.5, inocu crumu; at ph 6.15; it was 6.10. Neither ph studied.

crumu; at ph 6.15; it was 6.10. Neither ph studied.

acid inhibited L. plantarum at either ph studied. the C. jejuni cultures had a colony count of alimeted the colony of a number of a number of a number of a number of an area of area of an area of an area of an area of an area the c. Jejuni cultures had a colony count of Alimeto rate of the colony at ph 6.75; at the cfulminiate of inimitation of initial colony count of alimitation of initial colony count of alimitation of alimitation of initial colony count of alimitation of alimitat cru/mu; at pH 6.75; it was 6.54. All doses of Alimet® gave at pH 4.5. 0.83 g/L of reduction of circles inhibited C. jejuni at pH 4.5. of circles inhibited contains a 2.4 log reduction of circles i inhibited C. jejuni at pH 4.5. 0.83 g/L of Alimet growth at 2.4 log reduction of C. jejuni and 0.30 a/l approximately a 2.4 log reduction (0.108 a/L and 0.30 a/L approximately a 2.4 log of Alimet (0.108 a/L approximately a 2.4 log of Alimet (0.10 10 approximately a 2.4 log reduction of C. jejuni growth at 0.30 g/L) this pH. Lower dosages of Alimet® (0.108 g/L) reduction of C. jejuni growth at 0.30 g/L) this ph. Lower dosages of Alimet (U.108 g/L and 1.2 log reductions)
gave approximately 1.1 respectively. Formic acid demonstrated was shown against C.

respectively. Formic acid demonstrated was shown against C.

inhibition. No antibacterial activity formic acid demonstrated was shown against C. gave approximately 1.1 and 1.2 Log reductions and 1.2 log reductions able comparable respectively. 15 Innibition. No antibacterial activity was shown aging activity as shown aging activity was shown aging activity activity was shown aging activity was shown aging activity was shown aging activity activity was shown aging activity was shown activity activity was shown activity activity activity was shown activity activit n studled. These results are demonstrated graphically in Figures 1A, 1B, 1C, and 1D. Figures 2A, 2B, 2C, and 2D effects of formic acid and were inhihited the pH dependent hanteria atualism were inhihited demonstrate the pH the four hanteria atualism were inhihited demonstrate hand and all the pH the four hanteria atualism were inhihited demonstrate. demonstrate the ph dependent bacteria studied were in fact a demonstrate the four bacteria at nu of k 7k. in fact a limet. Alimet. Action formic acid or animate. 20 Alimet<sup>®</sup>. None of the four bacteria at ph of 6.75; hartaria by either the colony forming unit rount of each hartaria concentration studied. by either tormic acid or Alimet unit count increasing the most state the colony forming unit increasing the with the R. coli count increased. this pH, the colony torming unit count increasing the most, and increased, with the E. the least 25 L. plantarum increasing the least. 30 60

## EXAMPLE 2

[0623] The effect of higher dosages of Alimet® and formic acid on the colony count of S. enteritidis cultures was studied, following the procedure described in Example 1. The results obtained are given in Table 5 and illustrated in Figure 3.

### Table 5

Effect of formic acid and Alimet® on population of S. enteritidis after 4 hours at pH 4.5 and 6.75

initial colony count: 5.23 log cfu/mL

Acid (g/L)		pH = 4.5		pH = 6.75		
Alimet®	Formic	nic log cfu/mL Δ		log cfu/mL	Δ log	
_	_	5.15	-0.09	6.92	1.69	
1	_	5.02	-0.21	6.61	1.38	
3	_	4.76	-0.48	5.97	0.74	
5	_	2.37	-2.86	5.43	0.20	
_	1	5.01	-0.22	6.92	1.69	
_	3	4.55	-0.68	6.58	1.35	
	5	3.83	-1.41	6.10	0.87	

20

25

5

10

15

[0624] Prior to inoculation, the *S. enteritidis* cultures had a log cfu/mL of 5.15 at pH 4.5, and of 6.92 at pH 6.75. At pH 6.75, the addition of 5 g/L formic acid or 3 g/L Alimet® gave approximately a 1 log cfu/mL growth inhibition. An addition of 5 g/L Alimet® stops the growth of *S. enteritidis*. At pH 4.5, 5 g/L Alimet® reduces the growth of *S. enteritidis* by approximately 2.8 log cfu/mL. Lower concentrations of Alimet® gives a smaller effect. Formic acid at 5 g/L reduces the growth of *S. enteritidis* by approximately 1.3 log cfu/mL. Thus, at the dose ranges studied, the antibacterial effect of Alimet® against *S. enteritidis* is greater than that of formic acid. These results are demonstrated graphically in Figure 3.

## EXAMPLE 3

5

10

15

20

25

30

[0625] Combinations of Alimet® and formic acid were studied, following the procedure described in Example 1. The results obtained are given in Table 6 and illustrated in Figures 4A and 4B.

#### Table 6

Effect of formic acid and Alimet $^{(0)}$  on population of S.

enteritidis after 4 hours at pH 4.5 and 6.75

initial colony count: 5.15 log cfu/mL)

Acid (g/L)		pH = 4.5		pH = 6.75		
Alimet®	Formic	log cfu/mL Δ log log cf		log cfu/mL	Δ log	
_	_	4.99	-0.17	6.87	1.80	
3	_	4.76	-0.39	5.89	0.82	
5	_	2.07	-3.08	5.45	0.38	
_	3	4.57	-0.58	6.51	1.44	
_	5	3.94	-1.21	6.19	1.12	
0.75	2.25	4.78	-0.37	6.27	1.20	
1.25	3.75	4.01	-1.14	5.94	0.88	
1.50	1.50	4.73	-0.42	6.11	1.04	
2.25	0.75	4.78	-0.37	5.97	0.90	
2.50	2.50	2.48	-2.67	5.74	0.67	
3	5	1.15	-4.00	5.31	0.24	
3.75	1.25	2.11	-3.04	5.54	0.47	

[0626] Combinations of Alimet® and formic acid having a combined concentration of 5 g/L inhibit growth of S. enteritidis to a greater extent than do combinations having a combined concentration of 3 g/L. Three 5 g/L combinations were prepared, having Alimet®-to-formic acid ratios of 1:3, 1:1, and 3:1.

[0627] At pH 4.5, treatment with 3 g/L of Alimet® alone gave an approximately 0.4 log cfu/mL reduction in S. enteritidis growth. Treatment at that pH with 5 g/L of

formic acid gave an approximately 1.2 log cfu/mL reduction. Remarkably, treatment with a combination of 3 g/L Alimet® and 5 g/L formic acid gave a reduction of 4 log cfu/mL, which was higher than expected given the individual results with Alimet® and formic acid at those levels. The results obtained suggest that at pH 4.5, combinations of 2.5 g/L Alimet® and 2.5 g/L formic acid, and with 3 g/L Alimet® and 5 g/L formic acid may have a synergistic effect. The latter combination gives the best results of all tested combinations: at pH 4.5, this combination gives 4 log (almost complete) reduction of *S. enteritidis*.

### EXAMPLE 4

[0628] The effects of blends of organic acids (butyric, citric, formic, lactic, and propionic) and Alimet® on the colony counts of *E. coli* (ATCC 25922) grown in trypticase soy broth at 35°C according to the manufacturer's instructions were studied. Blends of organic acid:Alimet® of 2:1 and 5:1 were studied, at a total concentration (organic acid + Alimet®) of 6 g/L.

20

5

10

15

[0629] The pH of the solutions were originally adjusted to pH 5 by addition of HCl and/or NaOH as needed. Activated E. coli culture solutions were transferred to fresh soy broth twice at 24-hour intervals prior to before addition of the organic acid:Alimet® blend. E. coli culture solutions were centrifuged; pellet produced was resuspended with Butterfield buffer, and the resulting solutions were diluted to approximately 107 CFU E. coli/mL.

30

25

[0630] Bottles were inoculated with 100 µL of prepared bacterial suspension and an organic acid:Alimet® blend. Samples were taken after five and 24 hours of incubation, serially diluted and spread-plated on trypticase soy agar, and incubated at 35°C for 24 hours. Populations of *E. coli* are reported in **Table 7** below.

### Table 7

Effect of Alimet®/acid blends on E. coli populations in trypticase soy broth

initial colony count: 4.97 log cfu/mL

	Acid:Alimet®	original	log cfu/mL	
Acid	Acid ratio		t=4h	t=24h
Control		_	7.18	9.22
HCl	_	_	7.85	8.34
7	5:1	ca. 4.2	4.68	3.98
lactic	2:1	4.1	4.87	4.45
£	5:1	3.1	4.95	<1
formic	2:1	3.56	4.95	1.00
	5:1	4.75	6.38	8.59
citric	2:1	4.59	5.90	8.66
l- and an add an	5:1	4.62	4.77	3.70
butyric	2:1	4.6	4.85	3.80
	5:1	4.54	4.79	4.57
propionic	2:1	4.53	4.83	4.53

[0631] Blends with formic acid were the most effective among the tested blends to control E. coli at both 5:1 and 2:1 blends of formic acid:Alimet<sup>®</sup>. Upon prolonged exposure (after 5 hours), both ratio give nearly complete reduction of E. coli. Blends of lactic, butyric and propionic acids with Alimet<sup>®</sup> suppressed the growth of E. coli, but did not reduce the bacterial population in 24 hours.

## EXAMPLE 5

5

10

15

20

25

[0632] The effects of hydrochloric acid, formic acid, lactic acid, or Alimet $^{\otimes}$  on the colony counts of  $E.\ coli$  were studied. Amounts of formic acid, lactic acid, or Alimet $^{\otimes}$  were added to cultures of  $E.\ coli$ , grown in a soy

broth, at pH 4 or 7.3. The cultures were incubated, and increasing rimes The results are illustrated in Figure 5.

The results and Alimeto, decreased the colony and lactic acid, than hydrochloric acid herrer than hydrochloric acid.

Formic and lactic herrer than hydrochloric acid. process of the colony counts performed at increasing times. Formic and lactic acid, and Alimet, decreased the color in and Alimet, decreased the color of F and than hydrochloric acid. As in hydrochloric acid. As in hydrochloric acid. As in and hydrochloric acid. As in than hydrochloric acid. As in the hyd counts of E. coli better than hydrochloric acid. As in hydrochloric aci Example 1. Allmer snowed a petter reduction of E. cold
colony counts as compared to formic acids, and showed colony counts as compared to lactic acid. The effect of Alimet® on Salmonella in a meat, [0634] The effect of Alimets on Salmonella in a meat forth to the protocol sn-sal.

The effect of Alimets to the protocol sn-sal.

The effect of Alimets on Salmonella in a meat forth to the protocol sn-sal.

The effect of Alimets on Salmonella in a meat forth the protocol sn-sal.

The effect of Alimets on Salmonella in a meat forth the protocol sn-sal.

The effect of Alimets on Salmonella in a meat forth the protocol sn-sal.

The effect of Alimets on the protocol sn-sal.

The effect of Alimets of Alimets on the protocol sn-sal.

The effect of Alimets of Alimets of Alimets of Alimets meal premix was studied according to the protocol set tor (1979) 50-54).

Meal premix was snoeyenbos (Poultry Sci. 58 (1979) Inc., Easton. Mo. 100 Products. Easton. by smyser and snoeyenbos (Equitive Sci. Sci. Inc., Easton, in the products, aride products, ar Meat meal premix (Papillon Ag Products, Inc., Easton, Wu)

Meat meal premix (Papillon Ag Products, Inc., Easton, Inc., Easton, Wu)

The products, Inc., Easton, United States and 5 containing approximately 11% crude protein was use containing approximately 11% test sample for each assays. Ten grams of premix test sample for each assays. assays. Ten grams of premix test sample for each into a studied were measured into concentration of Alimet® studied were measured concentration (three renlination of alimet® studied were concentration (three renlination of alimet® studied were concentration (three renlination of alimet® studied were concentration of alimet® studied were concentration (three renlination of alimet®). EXAMPLE 6 sterile tube (three replicates per sample). Sterile water noisture

(1 mL) was added to each tube to assure adequate moisture

(2 mL) was added to each tube to assure adequate moisture level of (1 mL) was added to each tube to assure adequate level of was added to each tube to assure and moisture was was for salmonella multiplication. A final mark sample was for salmonella multiplication. for salmonella multiplication. A final molsture sample was achieved after inoculation. Fach transfer heart animing of 20% was achieved after 1 n mr. of a diluted rea heart animing of a diluted real heart animal molsture and heart animal molsture and heart animal molsture animal molsture of a diluted real heart animal molsture and heart animal molsture of a diluted real heart animal molsture and heart animal molsture of a diluted real heart animal molsture and heart animal molsture animal molsture and heart animal molsture animal molsture animal molsture animal molsture and heart animal molsture animal molst 20% was achieved after inoculation. Each test sample was protected after inoculation. Each test sample was achieved after inoculation. Each test sample was protected inoculated with the columns of a diluted TSB broth culture of a diluted TSB broth culture of inoculated with the columns of a diluted TSB broth culture of inoculated with the columns of a diluted TSB broth culture of inoculated with the columns of a diluted TSB broth culture of inoculated with the columns of a diluted TSB broth culture of inoculated with the columns of a diluted TSB broth culture of inoculated with the columns of a diluted TSB broth culture of a diluted TSB broth 10 inoculated with 1.0 ml of a diluted TSB broth culture of a capproximately approximately are a diluted TSB broth culture of a 10. cells/g as determined from spread plate counts of the mixed with a sterile and incubated and incubated at a counts of the mixed with a sterile mixed with a sterile and incubated at a counts of the counts of t culture). The inoculated samples were mixed with a sterile tool and incubated at 37°C for tongue blade or equivalent tool and incubated at tongue blade or the trial tongue blade of the trial 15 nuration of the trial. were determined using [10635] Lu6351 Salmonella counts were determined using days are counts were determined using salmonella counts were determined using and the same salium nalidixate at days are containing sodium nalidixate at days are counts were determined using counts were determined using days are counts were determined using and days are counts were determined using counts were determined using counts were determined using counts were determined using counts are containing sodium nalidixate at days are containing and counts were determined using counts are containing sodium are containing are containing are containing are contained using contained using containing are contained using containing brilliant green agar containing sodium nalidixate at days

brilliant green agar containing sodium nalidixate at tube and

brilliant green agar containing sodium nalidixate at tube and

transferred to 9 mi. aterile water

transferred to 9 mi. aterile water 1. 2 and 3. A 1 g test sample was taken trom each tube ar this test sample was the then are the transferred are the trans transferred to 9 min sterile water. This test then agitated to 9 min sterile water. This test then agitated then approximately 4 hours, then ailuted to 9 min sterile water. This test then agitated then agitated then approximately 4 hours, as another was serially diluted to the sample was then agitated to the sample was the sample was then agitated to the sample was serially diluted to the sample wa 20 the duration of the trial. Incubated at 4°C for approximately 4 nours, then agitated in an approximately 4 nours, then adiluted in an approximately 4 nours, then adiluted in an approximately 4 nours, then adiluted in a serially diluted in an approximately 4 nours, then adiluted in a serially diluted in an approximately 4 nours, then adiluted in a nours, the adiluted in adiluted in a nours, then adiluted in a nours, the adiluted in adiluted in a nours, the adiluted in a nours, the adiluted in adiluted in a nours, the adiluted in adiluted in a nours, the adiluted in a nours, the adiluted in adiluted in a nours, the adiluted in a nours, the adiluted in adiluted in a nours, the adiluted in adiluted in a nours, the adiluted in adiluted in adiluted in a nours, the adiluted in adiluted in adiluted in a nours, the adilute tor 60-yu seconds. Each test sample was serially diluted and lock undiluted and lilo and lilo and lilo diluted sample. The sample was serially diluted and lilo diluted sample. The sample was serially diluted and lilo diluted sample. The sample was serially diluted and lilo diluted sample. The sample was serially diluted and lilo diluted sample. The sample was serially diluted and lilo diluted sample. 1:100 and 1:1000 proportions, and 100 µL of undiluted sample, and 1:100 diluted sample, area area 1:10 diluted sample, hrilliant area area 1:10, hrilliant area area area 1:10, hrilliant area area area area area area area. 1:1000 diluted samples were plated on prilliant green agar recovery 25 30

of Salmonella for different levels of Alimet® is reported in Table 8 and Figure 7.

5

10

15

20

25

30

Table 8							
Recovery of Salmonella in meat meal premix with 20% moisture; 1:10 dilution							
Alimet®	Alimet®	% 5	Salmonell	a recove	red		
conc. (reported)	conc. (found)	day 0	day 1	day 2	day 3		
control	control (0%)		1990%	971%	267%		
0.275%	0.056%	100%	102%	67%	< 3%*		
0.18%	0.140%	100%	190%	65%	11%		
0.25%	0.188%	100%	476%	139%	25%		
0.36%	0.192%	100%	114%	62%	4%		
0.40%	0.220%	100%	343%	267%	53%		
0.69%	0.631%	100%	5%	< 3%*	< 3%*		
* below detect	ion limit						

[0636] The control sample (no Alimet® added) showed an initial steep increase in Salmonella population one day after inoculation, indicating multiplication of the bacteria. This multiplication was followed by a gradual decline in the bacterial counts on days 2 and 3. Results from the highest level of Alimet® tested suggest that Alimet® is bactericidal for Salmonella in meat and bone meal. As Figure 8 illustrates, Alimet® at this level gives results comparable to treatment with formic acid at 1.65% (15 kg/ton). See Liu, "Using Organic Acids to Control Salmonella in Poultry Production," Kemin Industries (Asia) Pte Limited, Singapore, available at http://www.kemin.com.

[0637] Alimet® in the range of 0.14-0.22% likewise showed anti-Salmonella effects, and does not appear to be dose-dependent in this range. However, each sample was from different batches of MBM, which may be responsible for the lack of dose-response. The initial multiplication seen in the control was significantly reduced upon addition of

Alimet<sup>®</sup> at these levels. The subsequent decline occurred faster for these lower levels of Alimet<sup>®</sup> compared to the control.

## EXAMPLE 7

5

10

15

20

25

30

35

[0638] Fungal growth in basal starter mash was studied in compositions supplemented with DLM or Alimet® as the methionine source. Total microbial growth was monitored by measuring carbon dioxide (CO<sub>2</sub>) formation in sealed vessels at 28°C over time. Measurement of CO<sub>2</sub> formation does not distinguish between bacterial and mold growth; however, the ability of mold to grow at much lower water activities, compared to bacteria, is well known, and both mold and bacteria play a part in feed degradation.

[0639] The technique of using a closed system and measuring  $CO_2$  formation has been verified as an approximation of the conditions found in grain storage bins. See, e.g., Muir et al., <u>Trans. ASAE</u>, 28(5) 1673-1675 (1985), the contents of which are hereby incorporated by reference in their entirety.

[0640] A mash starter mash feed (formulation shown in Table 9, below) was subdivided into three groups: basal (control), 0.2% DLM, or 0.2% Alimet<sup>®</sup>. The feed studied had no commercial mold inhibitors added, and is representative of a typical broiler feed.

[0641] Initial moisture of the feed was 10.8%. After the addition of Alimet® or DLM, the moisture of the samples was adjusted by the addition of 2%, 4%, or 6% sterile distilled water to promote mold growth, achieving three moisture groups: 83.2% dry matter/12.8% moisture; 85.2% dry matter/14.8% moisture; and 87.2% dry matter/16.8% moisture.

[0642] For each study, four replicate samples of each moisture group were mixed, and 600 g of the mixture was placed into 1L containers, sealed, and placed at  $28\,^{\circ}$ C in a temperature-controlled room. Draeger Detector tubes ( $CO_2$ -measuring, obtained from Fisher Scientific) were used to measure the developed  $CO_2$  at different days following vessel

sealing (two measurements were made per week). Draeger Short-Term Detector Tubes are glass tubes filled with inert carrier and an indicating reagent. The reagent produces a colorimetric indication in the presence of a particular gas  $(CO_2)$ . The concentration of gas is read directly from the discoloration on the tube's printed scale.

5

10

15

20

25

30

[0643] The specific mold species present were not identified. Statistical analysis was accomplished by using Duncan's multiple range test (SAS). Different letters on individual time points in Figures 9-11 indicate statistical differences of P < 0.05.

Table 9 Basal starter mash formulation	
Ingredient	% by weight of total mix
Corn	60.551
Soybean meal	32.254
Fat, animal	3.665
Dical940224PhosfromD (dicalcium phosphate)	1.861
Limestone	0.811
Novus Vitamin/Mineral premix manufactured by Trouw Nutrition (Highland, Illinois)	0.350
Salt	0.340
L-lysine HCl 78%	0.097
Threonine	0.051
Santoquin-mix6 Antioxidant preservative sold by Solutia Inc. (St. Louis, Missouri)	0.019
Copper Sulfate	0.003

[0644] As shown in Figure 9, Alimet® effectively inhibited mold growth for up to seven days at the highest moisture level tested (83.2% dry matter/16.8% moisture), while DLM was the least effective, and, in fact, showed mold growth within two days. In fact, DLM-treated starter

mash showed mold growth faster than basal mash (i.e., feed with no added methionine or methionine analog), and faster than the Alimet®-treated mash, for all moisture levels tested.

5

[0645] As demonstrated in Figure 10, Alimet®-treated feed showed a slower rate of mold growth in 85.2% dry matter/14.8% moisture feed, than the DLM-treated feed.

10

[0646] For feed having 87.2% dry matter/12.8% moisture, Alimet®-treated feed shows low mold levels for up to sixty days, while DLM-treated feed shows a sharp increase in mold growth after only twenty days (see Figure 11).

15

[0647] Figures 9, 10, and 11 each illustrate that DLM treated mash is more likely to develop mold than either methionine-deficient feed or feed treated with Alimet®, and that Alimet® was more effective in inhibiting mold growth than compared to untreated feed, or feed supplemented with DLM.

### EXAMPLE 8

20

[0648] The experiment described above in Example 7 was repeated with blends of 2.0 lb/ton, 1.5 lb/ton, 1.0 lb/ton or 0.5 lb/ton of 65% propionic acid and either 2% Alimet® or 2% DLM. The blends were prepared according to the matrix outlined in Table 8, below. The 65% propionic acid was buffered with ammonium hydroxide to a pH of 5.5.

	Table 10						
Ani	Antifungal blends of Alimet® or DLM with propionic acid						
Trial No.	Trial No. Alimet® Propionic D (%) (lb/ton) (						
1	0.2	_	_				
2	-	2	0.2				
3	_	1.5	0.2				
4	_	1.0	0.2				
5	_	0.5	0.2				
6	_	_	0.2				
7	0.2	2					
8	0.2	1.5	_				
9	0.2	1.0	_				
10	0.2	0.5	_				

15

10

5

[0649] Statistical analysis was accomplished by using Duncan's multiple range test (SAS). Different letters on individual time points in graphs indicate statistical differences of P<0.05.

20

[0650] As shown in Figure 12, basal diet having 85.2% dry matter and containing 0.2% Alimet® delayed the onset of mold growth by 5 days; the effect of 2.0 lb/ton, 1.5 lb/ton and 1.0 lb/ton 65% propionic acid is about 11 days, i.e, no significant difference was seen when propionic acid was added beyond 1 lb/ton.

25

[0651] Combinations of Alimet® and propionic acid were compared to feed treated with propionic acid alone, and the results are shown in Figures 13-15. The results indicate that, for all moisture levels studied, basal feed containing Alimet® plus propionic acid showed improved mold inhibition compared to feed containing only propionic acid.

#### EXAMPLE 9

[0652] The effect of formic, butyric and lactic acids on Salmonella populations in basal corn soy based broiler starter feed was studied to determine the levels of these acids required for complete bacteriacide.

[0653] Tests were carried out using 1 g feed (crumble ground) with 6% meat and bone meal ("MBM"). Aqueous 25% solutions of formic, butyric, and lactic acids were prepared. The acid solutions were added to the feed as indicated below; water (1 mL) and 150 mM HCl (1.8 mL) was added to bring the pH to 4.0. Naldixic Acid resistant Salmonella (provided by Dr. Stan Bailey, USDA/ARS, Athens, Georgia) (initial colony count = 40,000 cfu/g) was added, and the feed solutions were incubated at 37°C for 90 min. Each sample was diluted with 6 mL H<sub>2</sub>O, plated, and counted the following day. Colony counts are reported in Table 11 below.

т	a	h	7	0	1	1
_	Q.	_	_	_		_

Effect of formic, butyric or lactic acids on Salmonella populations in feed  $[\Delta \log = \log_{sample} - \log_{control}]$ 

Acid	g/L	vol. (µL)	final pH	log cfu/g	Δ log
control			4.47	5.0	
formic	2.5	10	4.28	4.3	0.7
	5.0	20	4.18	3.6	1.4
	7.5	30	4.1	1.0	4.0
	10	40	4	1.0	4.0
butyric	10	40	4.31	4.3	0.7
	30	120	4.17	1.0	4.0
	50	200	4.04	1.0	4.0
lactic	10	40	4.2	4.1	0.9
	30	120	3.92	1.0	4.0
	50	200	3.68	1.0	4.0
control			4.44		

5

10

15

[0654] Complete bacteriacide was seen at the two highest doses tested for all three acids (7.5 and 10 g/L for formic acid; 30 and 50 g/L for butyric and lactic acids).

### EXAMPLE 10

[0655] Following the procedure set forth in Example 9, the effect of blends of formic, butyric and/or lactic acids, with and without Alimet®, on Salmonella counts in basal corn soy based broiler starter feed (described in Table 9, above, crumble feed with 6% MBM) was studied. The blends studied are described in Table 12, and the results obtained in the *in vitro* study are reported in Tables 13-15.

Table 12							
Acid blend formulations (concentrations reported in g/L)							
Blend	Formic Acid	Lactic Acid	Butyric Acid	$ t Alimet^{ t  ext{@}}$			
A1	5	_		_			
A2	4	4	_	_			
A3	4	_	4	_			
A4	3	_	8	_			
<b>A</b> 5	3	4	4	<del>-</del>			
A6	3	8	_	_			
A7	2	_	12	<del>-</del>			
A8	2	4	8	_			
A9	2	8	4	_			
A10	2	12	_				
A11	1		16				
A12	1	4	12	_			
A13	1	8	8	_			

A14

A15

A16 A17 A18 A19 A20 A21	Acid	Acid — 4 8	20 16 12	
A17 A18 A19 A20		8	16	_
A18 A19 A20		8		
A19 A20			1 1/	_
A20		12	8	_
<del>-</del>	_	16	4	
		20		_
A22	5		_	1
A23	4	4	_	1
A24	4	_	4	1
A25	3	_	8	1
A26	3	4	4	1
A27	3	8	_	1
A28	2	_	12	1
A29	2	4	8	1
A30	2	8	4	1
A31	2	12	_	1
A32	1	_	16	1
A33	1	4	12	1
A34	1	8	8	1
A35	1	12	4	1
A36	1	16	_	1
A37	_	_	20	1
A38	<u>—</u>	4	16	1
A39	_	8	12	1
A40		12	8	1
A41	_	16	4	1
A42	_	20		1
A43	5	_	-	2.27

	Table 12, cont'd							
Blend	Formic Acid	Lactic Acid	Butyric Acid	Alimet®				
A45	4	_	4	2.27				
A46	3	_	8	2.27				
A47	3	4	4	2.27				
A48	3	8	_	2.27				
A49	2	<del>-</del>	12	2.27				
A50	2	4	8	2.27				
A51	2	8	4	2.27				
A52	2	12	_	2.27				
A53	1	_	16	2.27				
A54	1	4	12	2.27				
A55	1	8	8	2.27				
A56	1	12	4	2.27				
A57	1	16	_	2.27				
A58	_	_	20	2.27				
A59	_	4	16	2.27				
A60	_	8	12	2.27				
A61	_	12	8	2.27				
A62	_	16	4	2.27				
A63	_	20	_	2.27				

Table 13

Effect of formic/butyric/lactic blends without Alimet® on Salmonella populations in feed

	Salmonella populations in feed						
Blend	Final pH	log cfu/g	$\Delta$ log reduction				
A1	4.12	3.1	1.6				
A2	4.13	2.9	1.8				
A3	4.18	3.1	1.6				
A4	4.18	3.0	1.7				
<b>A</b> 5	4.18	3.2	1.5				
A6	4.15	2.6	2.1				
A7	4.15	2.8	1.9				
A8	4.18	2.8	1.9				
A9	4.16	2.5	2.2				
A10	4.12	1.8	2.9				
A11	4.16	2.7	2.0				
A12	4.17	2.7	2.0				
A13	4.16	2.8	1.9				
A14	4.14	2.9	1.8				
A15	4.1	2.6	2.1				
A16	4.16	2.7	2.0				
A17	4.21	2.7	2.0				
A18	4.18	2.5	2.2				
A19	4.16	1.7	3.0				
A20	4.13	2.7	2.0				
A21	4.05	1.8	2.9				
control	4.33	4.7	_				

# Table 14

Effect of formic/butyric/lactic blends with 1 g/L added Alimet® on Salmonella populations in feed

5

10

15

20

Blend	Final pH	log cfu/g	Δ log reduction
A22	4.09	3.5	1.0
A23	4.09	2.1	2.4
A24	4.09	2.9	1.6
A25	4.12	2.2	2.3
A26	4.11	2.3	2.2
A27	4.1	1.9	2.6
A28	4.11	2.3	2.2
A29	4.14	2.3	2.2
A30	4.1	1.9	2.6
A31	4.06	1.6	2.9
A32	4.15	2.4	2.1
A33	4.15	2.3	2.2
A34	4.15	1.4	3.1
A35	4.11	1.7	2.8
A36	4.06	1.8	2.7
A37	4.13	2.0	2.5
A38	4.16	2.0	2.5
A39	4.13	1.8	2.7
A40	4.12	1.8	2.7
A41	4.08	1.7	2.8
A42	4.06	1.7	2.8
control	4.33	4.7	0.2
control	4.36	4.5	

Table 15

Effect of formic/butyric/lactic blends with 2.27 g/L added Alimet® on Salmonella populations in feed

5

10

15

20

25

30

Alime	Alimet® on Salmonella populations in feed				
Blend	Final pH	log cfu/g	Δ log reduction		
A43	4.21	2.8	0.7		
A44	4.17	2.8	0.7		
A45	4.18	3.0	0.5		
A46	4.18	2.8	0.7		
A47	4.15	3.7	-0.2		
A48	4.11	2.0	1.5		
A49	4.19	2.7	0.8		
A50	4.19	2.8	0.7		
A51	4.16	2.8	0.7		
A52	4.13	2.4	1.1		
A53	4.2	2.8	0.7		
A54	4.2	2.7	0.8		
A55	4.14	2.3	1.2		
A56	4.13	1.7	1.8		
A57	4.08	1.0	2.5		
A58	4.21	2.3	1.2		
A59	4.23	2.7	0.8		
A60	4.17	2.0	1.5		
A61	4.14	2.0	1.5		
A62	4.12	0.7	2.8		
A63	4.07	0.7	2.8		
control	4.37	3.5	0		
control	4.45	3.5	_		

[0656] Addition of 1 g/L Alimet $^{\odot}$  to the blend gave improved results in sixteen of the blends tested.

[0657] The formulations of the blends used in Examples 11-13 are set forth in Table 16.

	Table 16											
	Organio	acid/Al	imet® ble	nd formul	ations							
Blend	Alimet®	Phos.1	Butyric	Formic	Lactic	Prop. <sup>2</sup>						
A64	30	30	20	_	20	_						
A65	30	_	_	50	_	20						
A66	20	20	12	30	18	-						
A67	10	10	<del>-</del>	75	-	5						
A68	30	35	15	-	20							
A69	30	5	_	55	_	10						
A70	20	20	12	_	18	30						
A71	10	5	5	75	_	5						
A72	_		_	50	_	50						
A73			_	75		25						
		i										

5

10

15

20

[0658] The effects of blends of organic acids on the colony counts of Salmonella in a corn soy based diet as set forth in Table 9, above, (DLM at 0.2%) were studied, following the procedure outlined in **Example 9**. The results are reported in Table 17.

Table 17 effect of acid blends on Salmonella populations in corn soy based diet

		after 60 min.			
Blend	g/kg	cfu/g <sup>1,2</sup>	log cfu/g	$\Delta$ log reduction	
cont	rol	40,000	4.6	_	
7.64	5	29,700	4.5	0.1	
A64	10	2,300	3.4	1.2	
7.65	5	3,100	3.5	1.1	
A65	10	40	1.6	3.0	
7.55	5	10,000	4.0	0.6	
A66	10	40	1.6	3.0	
7.67	5	3,000	3.5	1.1	
A67	10	40	1.6	3.0	
cont	rol	13,900	4.1	_	

10

15

		•					
			after 90 min.				
Blend	g/kg	cfu/g <sup>1,2</sup>	log cfu/g	$\Delta$ log reduction	final pH		
cont	rol	46,000	4.7	_	4.68		
7.64	5	19,300	4.3	0.4	4.45		
A64	10	2,400	3.4	1.3	4.32		
7.65	5	200	2.3	2.4	4.44		
A65	10	40	1.6	3.1	4.25		
3.55	5	13,500	4.1	0.6	4.37		
A66	10	40	1.6	3.1	4.22		
3.65	5	200	2.3	2.4	4.33		
A67	10	40	1.6	3.1	4.02		
cont	rol	38,800	4.6	-	4.68		

 <sup>40</sup> cfu/g (minimum detection level) reported when no Salmonella detected.
 Single reading for each treatment.

[0659] Three of the four blends tested showed complete bacteriacide at 10 g/kg (1%) application. Both the A65 and A67 blends showed significant bacteriacide at the lower application rate (0.5%)

## EXAMPLE 12

5

10

15

20

[0660] The effect of blends of organic acids on Salmonella counts were studied using model poultry and swine diets. Blends A70 and A71 were tested using the corn soy based diet set forth in Table 9, above, with DLM added at 0.2%. The model poultry diet was a corn soy based layer diet, no meat product; the effects of blends A68 and A69 were tested with this diet.

[0661] Blends were added to 1 g of feed sample. Salmonella (200  $\mu$ L, 40,000 cfu) were added to each feed sample, and mixed. The samples were incubated at room temperature, then diluted 1:10 with water and plated on BG plate. Results are reported in **Table 18**.

Table 18	T	ab	le	1	8
----------	---	----	----	---	---

effect of acid blends on Salmonella populations in corn soy based diet

h1 and ( /)		Final	aft	er 1 h	after 24 h	
blend	g/kg	Нq	cfu/g	log cfu/g	cfu/g	log cfu/g
cont (Diet		5.83	12,400	4.1	10,133	4.0
	2	5.53	15,000	4.2	3,300	3.5
770	5	5.32	3,900	3.6	1,200	3.1
A70	7.5	5.08	3,400	3.5	700	2.8
	10	4.94	1,500	3.2	100	2.0
	2	5.42	9,600	4.0	2,300	3.4
3.77	5	5.16	1,300	3.1	100	2.0
A71	7.5	4.84	200	2.3	100	2.0
	10	4.66	100	2.0	100	2.0

Table 18

effect of acid blends on Salmonella populations in corn soy based diet

hland a/ha		Final	after 1 h		after 24 h	
blend	g/kg	рH	cfu/g	log cfu/g	cfu/g	log cfu/g
cont (Diet		5.92	15,300	4.2	6,300	3.8
	2	5.70	10,400	4.0	5,600	3.7
7.60	5	5.67	6,300	3.8	4,200	3.6
A68	7.5	5.54	9,900	4.0	2,600	3.4
	10	5.36	8,300	3.9	1,300	3.1
	2	5.53	7,800	3.9	3,300	3.5
	5	5.29	3,600	3.6	1,200	3.1
A69	7.5	5.22	1,200	3.1	300	2.5
	10	5.08	1,000	3.0	100	2.0

5

10

[0662] The antibacterial effect of two organic acid/Alimet® blends were compared with blends containing formic and propionic acids, and with no Alimet®, following the procedure set forth in Example 12. Results after 90 minutes are reported in Table 19.

Table 19						
Blend	g/kg	cfu/g	log cfu/g	final pH		
cont	rol	29,400	4.5	4.54		
	2	140	2.1	4.56		
A65	5	80	1.9	4.45		
	10	1	0	4.32		
	2	900	3.0	4.57		
A67	5	1,100	3.0	4.49		
	10	1	0	4.25		
	2	2,100	3.3	4.5		
A72	5	90	2	4.39		
1	10	1	0	4.15		
	2	2,700	3.4	4.51		
A73	5	600	2.8	4.4		
	10	1	0	4.1		
cont	rol	30,650	4.5	4.68		

10

15

5

[0663] The antibacterial effect of two different batches of Alimet® on a corn soy diet (see **Table 9**, above) were compared. The first batch was of an unknown age and the second batch was freshly prepared (less than two weeks old). The protocol set forth in **Example 9** was used, and results are reported in **Table 20**.

#### Table 20

effect of two different batches of Alimet® on Salmonella populations in corn soy based diet after 90 min. incubation

111000001011				
Acid	g/kg added	cfu/g	log cfu/g	pН
contr	ol	6,850	3.8	4.80
	2.3	800	2.9	4.72
Alimet®	5.7	50	1.7	4.66
batch 1	8.5	120	2.1	4.58
	11.4	0	-	4.57
	2.3	3,900	3.6	4.68
Alimet®	5.7	300	2.5	4.54
batch 2	8.5	20	1.3	4.61
	11.4	20	1.3	4.57
contr	col	14,850	4.2	4.80

[0664] The first batch of Alimet® showed slightly improved bactericidal effects at lower concentrations. Both batches were bactericidal at higher doses.

## 15 EXAMPLE 15

5

10

20

[0665] The antibacterial effect of dry acids (fumaric, tartaric, and sorbic) alone and in combination with Alimet® blends were studied according to the protocol of Example 9. The formulations of the blends studied are reported in Table 21. The results after 90 minutes are reported in Table 22.

∆ log

reduction

2.8

3.0

0.3

1.4

4.4

0.8

2.2

	Table 21							
77		acid concentration (g/kg)						
Blend	Alimet®	Fumaric	Tartaric	Sorbic				
A74	10	0	0	0				
A75	0	10	0	0				
A76	0	0	10	0				
A77	0	0	0	10				
A78	5	5	0	0				
A79	5	0	5	0				
A80	5	0	0	5				

Table 22

log cfu/g

4.4

1.6

1.5

4.2

3.1

3.7

2.2

4.1

cfu/g

27900

40

30

14450

1150

0

4600

170

13900

10

5

15

20

25

30

# EXAMPLE 16

Blend

control

A74

A75

A76

A77

A78

A79

A80

control

[0666] The effect of formic acid on Lactobacillus plantarum was studied. As demonstrated in Example 1, addition of Alimet® at pH 3.5 to a bacteria-containing broth showed a clear lethal effect on L. plantarum at doses of 3 and 5 g/l. Comparable concentrations of formic acid (technical quality 85%, ex Franklin Products) were also studied and compared against Alimet®.

[0667] Fresh overnight culture L. plantarum in Brain Heart Infusion broth is used to inoculate medium 5 at a log

4.1 cfu/mL at pH 3.5. The tubes are incubated under oxygen reduced atmosphere for 6 hours at 37°C. Colony counts are performed according standard procedures. All analyses were performed in duplicate, and the results are reported in Table 23.

#### Table 23

Effect of Alimet® and formic acid on *L. plantarum* colony counts in broth (after 6 hours)

initial log cfu/mL = 5.61  $\Delta$  log = log<sub>6 hours</sub> - log<sub>initial</sub>

	556 nours51mctar					
Acid	g/kg	log cfu/mL	Δ log			
control		5.67	0.06			
Alimet®	3	3.02	-2.59			
	5	0.83	-4.78			
formic acid	3	3.19	-2.42			
	5	-0.30	-5.91			

#### EXAMPLE 17

5

10

15

20

[0668] Blends of various acids with Alimet® were studied at pH 4.5. The effect of these blends on S. enteritidis colony counts in broth were studied, using the protocol set out in Example 1. Results are reported in Tables 24-26.

	Table 24						
	Effects of blends of acids and Alimet® on S. enteritidis colony counts in broth						
Blend	Acid Acid g/L Alimet® g/L log cfu/mL						
		Control		5.04			
	Contr	ol (Alimet®)		-0.30			
A81		5	0	3.93			
A82	,	4.5	0.5	0.74			
A83	formic	4	1	0.24			
A84		3.75	1.25	0.15			
A85		3.5	1.5	-0.15			
A86		2.5	2.5	-0.30			
A87		5	0	4.44			
A88		4.5	0.5	3.56			
A89	hutimi a	4	1	1.70			
A90	butyric	3.75	1.25	1.44			
A91		3.5	1.5	-0.15			
A92		2.5	2.5	-0.30			
A93		5	0	4.74			
A94		4.5	0.5	4.40			
A95	citric	4	1	4.34			
A96		3.75	1.25	1.59			
A97		3.5	1.5	1.19			
A98		2.5	2.5	0.24			

Table 25

Effects of blends of acids or formaldehyde and Alimet® on S. enteritidis colony counts in broth

5

10

15

	5. enteritials colony counts in broth						
Blend	Acid	Acid g/L	Alimet® g/L	log cfu/mL			
		Control		5.02			
	Contr	ol (Alimet®)		0.00			
A99		5	0	4.68			
A100		4.5	0.5	4.65			
A101	£	4	1	4.43			
A102	fumaric	3.75	1.25	4.38			
A103		3.5	1.5	4.36			
A104		2.5 2.5		2.32			
A105		5	0	4.44			
A106	lactic	4.5	0.5	4.48			
A107		4	1	4.43			
A108		3.75	1.25	4.51			
A109		3.5	1.5	4.75			
A110		2.5	2.5	4.55			
A111		5	0	5.01			
A112		4.5	0.5	4.71			
A113	malic	4	1	4.77			
A114		3.75	1.25	4.91			
A115		3.5	1.5	4.96			
A116	l	2.5	2.5	4.89			

Table 26

Effects of blends of acids or formaldehyde and Alimet® on S. enteritidis colony counts in broth

	S. enteritidis colony counts in broth					
Blend	Acid	Acid g/L	Alimet® g/L	log cfu/mL		
	Con		4.95			
	Control	(Alimet®)		0.63		
A117		5	0	4.34		
A118		4.5	0.5	4.34		
A119	n maniania	4	1	4.10		
A120	propionic	3.75	1.25	3.76		
A121		3.5	1.5	3.30		
A122		2.5 2.5		0.87		
A123		5	0	4.68		
A124	phosphoric	4.5	0.5	4.67		
A125		4	1	4.56		
A126	phosphoric	3.75	1.25	4.62		
A127		3.5	1.5	4.48		
A128		2.5	2.5	3.30		
A129		5	0	-0.30		
A130		4.5	0.5	-0.30		
A131	£	4	1	-0.30		
A132	formaldehyde	3.75	1.25	-0.30		
A133		3.5	1.5	-0.30		
A134		2.5	2.5	-0.30		

25

30

5

10

15

20

[0669] Phosphoric, fumaric, lactic, malic and propionic acids do not show a significant inhibitory effect at 5 g/l. Blends of these acids with Alimet® gave similar results, except for the 50:50 blend of fumaric and Alimet®, which gave greater than 2 log reduction in colony counts compared to 5 g/L of fumaric alone, and the 50:50 blend of phosphoric and Alimet®, which gave an approximately 1.3 log reduction compared to 5 g/L of phosphoric alone.

[0670] Blends of formic acid and Alimet® performed more favorably than formic acid alone for all the blends studied. Similarly, blends of butyric acid and Alimet®, and citric acid with Alimet®, gave improved bactericidal effects with increasing proportion of Alimet® added.

#### EXAMPLE 18

5

10

15

20

25

[0671] The antibacterial effect of acid blends was studied according to the protocol of Example 9. Phosphoric acid (75%) was obtained from Astaris (St. Louis, MO), lot # TK60. L-lactic acid (80%) was obtained from Purac America (Lincolnshire, IL), batch # 015703-A. Butyric acid (99+%) was obtained from Aldrich Chemical Co. (Milwaukee, WI), batch # 0.5110A. The formulations of the blends studied are reported in Table 27. The results are reported in Table 28.

	Table 27							
Blend	a	acid formulations (% of total)						
Brend	Alimet®	Lactic	Phosphoric	Butyric				
A135	0.33	0.67	_	_				
A136	0.317	0.633	0.05					
A137	0.267	0.533	0.20	_				
A138	0.25	0.50	0.25	_				
A139	0.33	0.33	_	0.33				
A140	0.317	0.317	0.05	0.317				
A141	0.267	0.267	0.20	0.267				
A142	0.25	0.25	0.25	0.25				
A143	0.33		_	0.67				
A144	0.317	_	0.05	0.633				
A145	0.267		0.20	0.533				
A146	0.25	_	0.25	0.50				

	Table 28					
Blend	cfu/g	log cfu/g	final pH			
control	7,900	3.9	4.86			
A135	20	1.3	4.49			
A136	20	1.3	4.39			
A137	340	2.5	4.52			
A138	160	2.2	4.44			
A139	80	1.9	4.50			
A140	20	1.3	4.49			
A141	160	2.2	4.42			
A142	160	2.2	4.43			
A143	160	2.2	4.48			
A144	40	1.6	4.48			
A145	240	2.4	4.53			
A146	20	1.3	4.42			
control	22,100	4.3	4.73			

5

10

15

20

25

[0672] The effects of blend A71 at neutral pH were tested. The blend was added to 1 g of feed sample. Salmonella (40,000 cfu (65ul)) was added to each 1 g sample, mixed, and incubated at room temperature. Following incubation, samples were diluted 1:10 with water and plated on a BG plate.

[0673] Two diets were studied, as shown in Table 29. Results are reported in Table 30.

	Table 29				
	Diet 1 (swine diet)				
	ingredients	% of total			
	corn	51.60			
5	SBM, 48	30			
	DairyLac 80	8.50			
	Menhaden fish meal (Select)	3.98			
	Choice white grease	3.00			
	Dicalcium Phosphate	1.24			
10	Limestone	0.34			
	Lysine	0.26			
	DL-Methionine	0.13			
	Threonine	0.16			
	Vitamins, TMs, Salt and Mecadox	0.93			
15	Corn Starch	to 100			
	Diet 2 (broiler diet)				
	ingredients	% of total			
	corn	60.551			
	SBM	32.254			
20	Fat, animal	3.665			
	Dicalcium Phosphate	1.861			
	Limestone	0.811			
	Vitamin/Mineral premix	0.350			
	Salt	0.340			
25	L-lysine HCl 78%	0.097			
	Threonine	0.051			
	Satoquin-mix6	0.019			
	Copper Sulfate	0.003			
	DL-methionine	0.2			

Table 30							
Blend tested: A71							
	1 hou	r	24 ho	ırs	48 ho	ırs	final
g/kg	cfu/g	log	cfu/g	log	cfu/g	log	рН
Diet 1							
control	27,700	4.4	6,432	3.8	2,530	3.4	5.83
2	4,320	3.6	80	1.9	40	1.6	5.52
5	3,840	3.6	40	1.6	0	_	5.21
7.5	3,080	3.5	80	1.9	0		5.01
10	1,560	3.2	0	-	0		4.87
Diet 2							
control	25,000	4.4	6,160	3.8	2,570	3.4	6.06
2	8,080	3.9	40	1.6	40	1.6	5.67
5	7,000	3.8	40	1.6	40	1.6	5.31
7.5	4,400	3.6	40	1.6	0		5.1
10	900	3.0	0	_	0	_	4.82

5

10

15

20

[0674] Following the protocol of Example 19, the effects of blend A69 at neutral pH were tested. Formic acid (85%) was obtained from BASF Corporation (Mt. Olive, NJ), product # 019723, lot # 87656216KO. Two diets were studied, as shown in Table 31. Results are reported in Table 32.

	Table 31					
	Diet 3					
	ingredients	% of total				
	soybean meal	33.40				
5	corn	32.85				
	wheat hard red	20.00				
	organic peas meal	5.00				
	fat, animal	4.80				
	dicalcium phophate	1.81				
10	limestone	0.98				
	salt	0.43				
	Vitamin/Mineral premix	0.35				
	Threonine	0.10				
	Avizyme1502	0.10				
15	Santoquin-mix6	0.02				
	Coban 60	0.05				
	copper sulfate	0.00				
	Diet 4					
	ingredients	% of total				
20	corn	60.50				
	soybean meal	28.46				
	limestone	7.76				
	Dicalcium Phosphate	1.63				
	animal fat	1.00				
25	Vitamin/Mineral premix	0.35				
	salt	0.26				
	Santoquin-mix6	0.02				
	copper sulfate	0.00				
	choline Cl-60%	0.00				

Table 32							
	blend tested: A69						
/1	1 hou	r	24 ho	urs	48 ho	urs	final
g/kg	cfu/g	log	cfu/g	log	cfu/g	log	рН
Diet 3							
control	68,000	4.8	2,470	3.4	880	2.9	6.05
2	5,600	3.7	120	2.1	40	1.6	5.67
5	8,900	3.9	40	1.6	40	1.6	5.46
7.5	4,600	3.7	40	1.6	80	1.9	5.28
10	10,600	4.0	40	1.6	0	1	5.13
Diet 4							
control	68,000	4.8	2,630	3.4	1,380	3.1	5.99
2	8,600	3.9	0	_	0	_	5.8
5	900	3.0	40	1.6	0	_	5.55
7.5	2,600	3.4	200	2.3	0	_	5.37
10	2,700	3.4	0		40	1.6	5.12

5

10

15

20

[0675] Following the protocol of Example 9, the effects of blends of Alimet®, lactic acid, formic acid, and/or butyric acid were studied. Blend formulations are set forth in Table 33. Up to five replicates were performed, and results are reported in Tables 34 and 35.

	Table 33										
	acid formulations (g/kg)										
Blend	A¹	L²	F <sup>3</sup>	B <sup>4</sup>		Blend	A¹	L²	$\mathbf{F}^3$	B <sup>4</sup>	
A147	0	0	0	10		A162	0	0	6.7	3.3	
A148	2	0	0	8		A163	2	0	5.3	2.67	
A149	7.5	0	0	2.5		A164	7.5	0	1.67	0.8	
A150	3.3	0	0	6.7		A165	0	10	0	0	
A151	2	2.67	0	5.33		A166	2	8	0	0	
A152	7.5	0.8	0	1.67		A167	7.5	2.5	0	0	
A153	0	0	3.3	6.7		A168	0	6.7	3.3	0	
A154	2	0	2.67	5.3		A169	2	5.3	2.67	0	
A155	7.5	0	0.8	1.67		A170	7.5	1.67	0.8	0	
A156	0	6.7	0	3.3		A171	0	3.3	6.7	0	
A157	2	5.3	0	2.67		A172	2	2.67	5.3	0	
A158	7.5	1.67	0	0.8		A173	7.5	0.8	1.67	0	
A159	0	3.3	3.3	3.3		A174	0	0	10	0	
A160	2	2.67	2.67	2.67		A175	2	0	8	0	
A161	7.5	0.8	0.8	0.8		A176	7.5	0	2.5	0	
1 Alim											

Alimet®
 Lactic acid
 Formic acid
 Butyric acid

	Table 34							
Trial:	P.A.	verage	9	1	2	3	4	
Blend	cfu/g	log	Δ log	cfu/g	cfu/g	cfu/g	cfu/g	
control	49,200	4.7	_	58,200	43,400	46,000	N/A	
A147	18,800	4.3	0.4	22,200	15,400	N/A	N/A	
A148	15,700	4.2	0.5	19,800	11,600	N/A	N/A	
A149	70	1.8	2.8	80	200	0	0	
A150	12,300	4.1	0.6	1,200	23,400	N/A	N/A	
A151	6,000	3.8	0.9	8,200	3,800	N/A	N/A	
A152	60	1.8	2.9	40	40	120	40	
A153	1,600	3.2	1.5	3,200	0	N/A	N/A	
A154	20	1.3	3.4	40	40	0	0	
A155	10	1.0	3.7	40	0	0	0	
A156	11,300	4.1	0.6	6,000	16,600	N/A	N/A	
A157	2,900	3.5	1.2	1,000	4,800	N/A	N/A	
A158	400	2.6	2.1	800	0	N/A	N/A	
A159	0	_	4.7	0	0	0	0	
A160	0	_	4.7	0	0	0	0	
A161	20	1.3	3.4	40	40	0	0	
control	63,133	4.8	_	56,800	64,000	68,600	N/A	
N/A = re	plicate	not p	erforme	d				

	Table 35							
Trial:	Α·	verage	9	1	2	3	4	
Blend	cfu/g	log	Δ log	cfu/g	cfu/g	cfu/g	cfu/g	
control	25,666	4.4	_	23,200	25,800	28,000	N/A	
A162	0	_	4.4	0	0	0	0	
A163	0	_	4.4	0	0	0	0	
A164	0	_	4.4	0	0	0	0	
A165	5,100	3.7	0.7	4,000	6,200	N/A	N/A	
A166	150	2.2	2.2	120	120	120	240	
A167	30	1.5	2.9	40	80	0	0	
A168	0	_	4.4	0	0	0	0	
A169	0	_	4.4	0	0	0	0	
A170	0	_	4.4	0	0	0	0	
A171	0	_	4.4	0	0	0	0	
A172	0	_	4.4	0	0	0	0	
A173	0		4.4	0	0	0	0	
A174	0	_	4.4	0	0	0	0	
A175	0	_	4.4	0	0	0	0	
A176	0	_	4.4	0	0	0	0	
control	20000	4.3		20200	19800	N/A	N/A	
N/A = re	plicate	not p	erforme	d				

5

10

15

20

25

[0676] Following the protocol of Example 9, the effects of blends of Alimet®, lactic acid, propionic acid (99%, obtained from Sigma Chemical Co., St. Louis, MO, lot P-1386), and/or butyric acid were studied. Blend formulations are set forth in Table 36. Up to five replicates were performed, and results are reported in Tables 37 and 38.

	Table 36									
	acid formulations (g/kg)									
Blend	A <sup>1</sup>	L <sup>2</sup>	P <sup>3</sup>	B <sup>4</sup>		Blend	A <sup>1</sup>	L <sup>2</sup>	$\mathbf{P}^3$	B <sup>4</sup>
A177	0	0	0	10		A193	2	0	5.3	2.67
A178	2	0	0	8		A194	7.5	0	1.67	0.8
A179	7.5	0	0	2.5		A195	0	10	0	0
A180	0	3.3	0	6.7		A196	2	8	0	0
A181	2	2.67	0	5.3		A197	7.5	2.5	0	0
A182	7.5	0.8	0	1.67		A198	0	6.7	3.3	0
A183	0	0	3.3	6.7		A199	2	5.3	2.67	0
A184	2	0	2.67	5.3		A200	7.5	1.67	0.8	0
A185	7.5	0	0.8	1.67		A201	0	3.3	6.7	0
A186	0	6.7	0	3.3		A202	2	2.67	5.3	0
A187	2	5.3	0	2.67		A203	7.5	0.8	1.67	0
A188	7.5	1.67	0	0.8		A204	0	0	10	0
A189	0	3.3	3.3	3.3		A205	2	0	8	0
A190	2	2.67	2.67	2.67		A206	7.5	0	2.5	0
A191	7.5	0.8	0.8	0.8		A207	10	0	0	0
A192	0	0	6.7	3.3						

<sup>&</sup>lt;sup>1</sup> Alimet<sup>®</sup>
<sup>2</sup> Lactic acid
<sup>3</sup> Propionic acid
<sup>4</sup> Butyric acid

Table 37							
Trial:	A	verage	verage		2	3	
Blend	cfu/g	log	Δ log	cfu/g	cfu/g	cfu/	
control	20,067	4.3		17,200	18,800	24,2	
A177	6,333	3.8	0.5	5,400	7,000	6,60	
A178	500	2.7	1.6	400	600	N/A	
A179	400	2.6	1.7	200	600	N/A	
A180	1,400	3.1	1.2	1,400	1,400	N/A	
A181	1,500	3.2	1.1	2,000	1,000	N/A	
A182	100	2.0	2.3	200	0	N/A	
A183	6,000	3.8	0.5	9,600	2,400	N/A	
A184	6,900	3.8	0.5	8,800	5,000	N/A	
A185	1,300	3.1	1.2	1,400	1,200	N/A	
A186	4,100	3.6	0.7	5,400	2,800	N/A	
A187	2,400	3.4	0.9	600	4,200	N/A	
A188	400	2.6	1.7	200	600	N/A	
A189	4,700	3.7	0.6	1,000	8,400	N/A	
A190	7,300	3.9	0.4	7,800	6,800	N/A	
A191	300	2.5	1.8	600	0	N/A	
control	18,733	4.3		20,000	22,800	1340	

	Table 38								
Trial:	A	verage	<b></b>	1	2	3	4		
Blend	cfu/g	log	Δ log	cfu/g	cfu/g	cfu/g	cfu/g		
control	7,800	3.9	_	9,800	7,200	6,400	N/A		
A192	6,100	3.8	0.4	6,600	5,600	N/A	N/A		
A193	2,600	3.4	0.8	2,200	3,000	N/A	N/A		
A194	25	1.4	2.8	50	50	0	0		
A195	400	2.6	1.6	800	0	N/A	N/A		
A196	300	2.5	1.7	200	400	N/A	N/A		
A197	0	_	4.2	0	0	0	0		
A198	2,400	3.4	0.8	1,000	3,800	N/A	N/A		
A199	600	2.8	1.4	0	1,200	N/A	N/A		
A200	0	_	4.2	0	0	0	0		
A201	3,800	3.6	0.6	3,200	4,400	N/A	N/A		
A202	900	3.0	1.2	1,000	800	N/A	N/A		
A203	0	_	4.2	0	0	0	0		
A204	3,500	3.5	0.6	5,400	1,600	N/A	N/A		
A205	100	2.0	2.2	200	0	N/A	N/A		
A206	0	_	4.2	0	0	0	0		
A207	0	-	4.2	0	0	0	0		
control	30,600	4.5	_	41,600	19,400	30,800	N/A		
	N/	/A = 1	replicat	e not pe	erformed				

[0677] The ability of Alimet® and DLM to function as palatants for dog and cat food was studied. Alimet® and DLM were added into premium-type dog and cat food in the mixer to test the acceptance of the food compared to food lacking either supplement. The food used comprised good quality protein, and was high in CP and fat. A premium palantant was also added to the food. Alimet® or DLM was added into the mixer prior to extrusion. The formulation of the feline diet is described in Table 39, and of the canine diet in

Table 40. The Alimet®/DLM supplementation levels, and intake ratios, are described in Table 41. The intake ratio describes the relative incidence of selecting one food over another.

[0678] For the canine study, twenty-one dogs were used (seven small, seven medium, and seven large dogs). For the feline study, twenty mature cats were used. The animals were given two choices of food, placed in separate bowls. Over a two-day period, the dogs were given access to the food for 30 minutes; cats had access for 22 hours. The food chosen and consumed first was observed.

Table :	39
Feline D	iet
Ingredient	% of total diet
Corn	14.7
Poultry byproduct (low ash)	20
Soybean Meal	12.5
Corn gluten meal	12.2
Meat and Bone meal	4
Animal fat	11.4
Rice brewer's	19.4
Flavor	2
Fish meal	2
Dried eggs	0.5
Salt	0.5
KCl	0.5
Vitamins	0.2
Choline	0.1
Taurine	0.1
Tocopherol	0.05
Trace Minerals	0.05

Table	e 40
Canine	Diet
Ingredient	% of total diet
Corn	33.0
Barley	15.0
Poultry byproduct (low ash)	13.0
Poultry byproduct	12.0
Soybean Meal	9.0
Animal fat	8.0
Rice brewer's	5.0
Flavor	2.0
Dried eggs	1.0
Salt	0.5
Limestone	0.36
Vitamins	0.2
Choline	0.1
Tocopherol	0.05
Trace Minerals	0.05

	Table 41	
	Foods compared	Intake Ratio
	Canine	
	0.05% Alimet vs. control	2.07:1
5	0.10% Alimet vs. control	5.58:1
	0.15% Alimet control	5.13:1
	0.10% DLM vs. control	5.32:1
	0.15% DLM vs. control	4.95:1
	0.10% Alimet vs. 0.05% Alimet	2.54:1
10	0.15% Alimet vs. 0.05% Alimet	1.99:1
	0.15% Alimet vs. 0.10% Alimet	2.54:1
	0.15% DLM vs. 0.10% DLM	1.57:1
	0.10% DLM vs. 0.10% Alimet	1.05:1
	0.15% DLM vs. 0.15% Alimet	2.5:1
15	Feline	
	0.20% Alimet® vs. control	1.67:1
	0.25% Alimet® vs. control	1.91:1
	0.30% Alimet® vs. control	1.85:1
	0.25% DLM vs. control	1.87:1
20	0.30% DLM vs. control	1.63:1
	0.25% Alimet® vs. 0.20% Alimet®	1.30:1
	0.30% Alimet® vs. 0.20% Alimet®	1.26:1
	0.30% Alimet® vs. 0.25% Alimet®	1.16:1
	0.30% DLM vs. 0.25% DLM	1.04:1
25	0.25% DLM vs. 0.25% Alimet®	1.24:1
	0.30% DLM vs. 0.30% Alimet®	1.47:1

30

[0679] Acceptance of food containing Alimet® or DLM was studied to evaluate dietary consumption under no-choice conditions. Food was offered to the animals (18 dogs: 6 small, 6 medium, 6 large; 15 cats) for one week. The urine pH of six of the cats was also monitored.

[0680] The diets described in Tables 39 and 40 above were used. Diets were supplemented with Alimet® or DLM (0.1% for the canine study, 0.25% for the feline study). Additional urine pH tests were carried out with 0.3% Alimet®.

[0681] Results of the urine pH experiments are given in Tables 42 and 43. Results of the acceptance text are given in Table 44.

	Table 42							
Cat No.	Urine pH							
cat No.	Control	Alimet 0.25%	DL Met 0.25%					
452	6.35	6.45	6.39					
453	6.44	6.2	6.38					
457	6.29	6.56	6.39					
460	6.25	6.03	6.36					
465	6.22	6	6.1					
475	6.56	6.44	6.36					

	Table 43						
Cat No.	U	rine pH					
cac No.	Control	Alimet® 0.3%					
450	6.20	6.03					
453	6.44	6.10					
465	6.04	5.53					
468	6.40	5.73					
469	6.68	6.31					

10

5

15

	Table 44							
Supplemen	tation	Total grams consumed						
leve	1	control	supplemented food					
Canine								
	0.05%	5,046	10,420					
Alimet®	0.1%	2,101	11,714					
	0.15%	2,419	12,978					
DIM	0.1%	2,114	11,244					
DLM	0.15%	2,734	13,542					
Feline								
	0.2%	1,111	1,853					
Alimet®	0.25%	955	1,827					
	0.3%	1,003	1,858					
DIM	0.25%	985	1,842					
DLM	0.3%	1,078	1,754					

5

[0682] In view of the above, it will be seen that the several objects of the invention are achieved and other advantageous results attained.

15

[0683] As various changes could be made in the above feed rations and methods without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawing shall be interpreted as illustrative and not in a limiting sense.

20

[0684] When introducing elements of the present invention or the preferred embodiment(s) thereof, the articles "a," "an," "the," and "said" are intended to mean that there are one or more of the elements. The terms "comprising," "including," and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

25

[0685] Unless otherwise specified, amounts expressed as percentages are in percent by weight.